Effect of Melatonin and Meta-topolin on plant hormones, proline content and enzymes activity in grape branches of Crimson seedless cultivar grown under salt stress *in vitro*

moshtak jmal Jawad1 , Makki N. Nayyef2

[1-1]Department of Horticulture and Landscape , Faculty, of Agriculture, AL-Qasim Green University, Babylon, Iraq.

author e-mail: moshtak.jmal@agre.uoqasim.edu.iq Corresponding[1]

[2]E-mail: makki@agre.uoqasim.edu.iq

Abstract

. The study was conducted in the Plant Tissue Culture Laboratory / College of Agriculture / Al-Qasim Green University / Department of Horticulture and Landscape Engineering, to conduct tissue culture experiments. During the year 2024-2025. To study the effect of Melatonin and Meta-topolin on plant hormones, proline content and enzyme activity in Crimson seedless grape branches grown under salt stress in vitro. The experiment included two factors, the first factor is sodium chloride at three different concentrations (0, 75, 150) mmol. L^{-1} and the second factor is growth regulators (Melatonin, Meta-topolin) at three concentrations (0.4 and 0.8 MEL, Metato 1) mg. L^{-1} and the interaction of factors on the content of plant hormones, proline content and total enzyme activity (catalyst, peroxidase) in the branches of European Crimson seedless grape. The results showed significant negative effects of salt stress on the content of plant hormones in the branches of the plants, as the treatment without adding NaCl was superior by giving the highest rate of branches content of auxin and cytokinin, which reached (151.12, 89.33 µg/kg fresh weight), respectively, compared to the 150 mmol. L^{-1} treatment, which gave the lowest rate of the mentioned characteristics. Significant positive effects were also shown on the activity of total enzymes (catalase and peroxidase) and the content of the amino acid proline in the branches of the plants, as the 150 mmol. L^{-1} treatment of NaCl was superior by giving the highest rate of activity of total enzymes (catalase and peroxidase) and the content of the amino acid proline, which reached (5.029, 67.29, 49.82 µmol/g fresh weight/min), respectively, compared to the 0 NaCl treatment, which gave the lowest rate of the mentioned characteristics. While the results of the study revealed that the plants adapted to the influence of growth regulators (Melatonin, Meta-topolin) showed a clear enhancement in the content of plant hormones, the activity of total enzymes (catalyst and peroxidase), and the content of the amino acid proline in the branches of the plants, as the 0.8M + Mt1 mg. L^{-1} treatment outperformed by giving the highest rate in the content of branches of auxin and cytokinin, the activity of total enzymes (catalyst and peroxidase), and the content of the amino acid proline, which reached (149.6 µg/kg fresh weight, 106.87 µg/kg fresh weight, 4.393 µmol/g fresh weight/min, 55.74 absorption units/g fresh weight, 47.24 µg. g^{-1} fresh weight), respectively, compared to the other treatments that gave the lowest rate of the mentioned characteristics. The results of the experiment showed a clear effect of the interaction between salt stress and growth regulators (Melatonin, Meta-topolin) on the content of plant hormones, total enzyme activity (catalyst and peroxidase), and the content of the amino acid proline in the branches of the plants, as the treatment of 0 mmol. L^{-1} of sodium chloride with the addition of growth regulators 0.8M + Mt1 mg. L^{-1} outperformed by recording the highest rate in the branches' content of auxin and cytokinin, reaching (171.10, 114.50 µg/kg fresh weight), compared to the interaction treatment of 150 mmol. L^{-1} of NaCl without adding growth regulators, which recorded the lowest rate in the mentioned characteristics. The interaction

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treatment of 150 mmol. L^{-1} of NaCl with the addition of growth regulators 0.8M + Mt1 mg L^{-1}, the highest activity rate of total enzymes (catalase and peroxidase) and the content of the amino acid proline reached (6.150 µmol/g fresh weight/min, 80.12 absorption units/g fresh weight, 55.15 µg. g^{-1} fresh weight) respectively, compared to the control treatment in the absence of growth regulators, which recorded the lowest peak rate for the mentioned characteristics.

Keywords: salt stress, crimson seedless, Melatonin, Meta-topolin, *in vitro*.

1-Introduction

: Grapes *Vitis vinifera* L., from the Vitaceae family, are grown worldwide and distributed across different climatic zones according to the adaptation of their varieties (subtropical, warm temperate and cold regions, and according to the varieties). The area planted with grapes was estimated at about 7.5 million hectares, planted with more than ten thousand grape varieties in the world (OIV, 2017). Global production reached 72 million tons in 2023 (FAO, 2024). External conditions are a major factor in determining the quality of grapes, because the growth, productivity, and quality of grape fruits are directly related to the high salinity of irrigation water (salt stress) and indirectly (changes in soil properties). Given the importance of the grape crop, it has received special attention in its cultivation and production (Al-Taey and Al-Ameer, 2023). The problem of salinity is a determining factor in the expansion of cultivated areas. Scientific studies have shown that different types of fruit and their related varieties vary in their tolerance to various environmental stresses, including the problem of salinity, as grape varieties vary in showing less changes in responses to stresses, coinciding with the high concentration of chloride ions in the soil, and it is considered An indicator of better ion exclusion capacity (Reta et al., 2024). Therefore, selecting varieties more tolerant to these stresses represents a crucial strategy to address the soil salinity prevalent in large areas of the world. This includes selecting the most salt-tolerant grape varieties for use in breeding and cultivation programs. Plant tissue culture techniques have been used in specialized studies for this purpose to study the cellular response to salt stress and evaluate the tolerance of the studied plant varieties to salt stress conditions. These techniques allow researchers to expose large numbers of cells to stress factors in small spaces and for short periods. In addition, they eliminate the influence of uncontrolled external conditions that usually accompany the study of the effect of the studied factor. Harsh environmental conditions (temperature, salinity, and water scarcity) can have a devastating impact on plant growth and productivity, potentially leading to the collapse of entire ecosystems (Zandalinas et al., 2020). Some experimental strategies, such as external treatments, are currently being applied in research to stimulate the intrinsic resistance of grape plants to stresses, including the use of growth regulators, such as growth regulators. Melatonin (N-acetyl-5-methoxytryptamine) and the growth regulator Meta-topolin were added to the tissue culture medium to stimulate shoot proliferation in grapevines. Xu and Yao (2019) tested the effect of salinity at 100 mmol L^{-1} of NaCl and Melatonin at 50 mg L^{-1} on the germination and growth of Crimson seedless grapevines. Vineyards treated with NaCl for 3 weeks showed significant wilting, and leaves exhibited necrotic phenotypes. Adding Melatonin at 50 mg L^{-1} in the presence of salt enhanced the grapevines' tolerance to NaCl and resulted in a significant increase in germination efficiency. Montanaro et al. (2022) tested the salinity tolerance of grapes in Vitis vinifera grapes at 100 mmol L^{-1} of NaCl and synthetic cytokinin at 80 mg L^{-1}. mg. L^{-1}. Salt-treated grapevines showed significant stress, compared to grapevines treated with synthetic cytokinin under salt stress, which showed sustained bud growth and improved

vine performance. It was observed that the type and concentration used directly affected branch multiplication in grape tissue culture, as shown in various studies on grape plants (Reiter et al., 2015; Arnao and Hernandez-Ruiz, 2015). The most prominent of these cultivars is Crimson seedless, a hybrid cultivar developed from crossing Vitis vinifera 'Emperor' and USDA Selection C33-199. 'Crimson Seedless' is a high-yielding commercial cultivar in California, characterized by bright red, firm fruits with excellent eating properties, flavor, and high nutritional value (Creasy, 2018). Due to the widespread problem of salinity in soil and irrigation water in Iraq, which limits the cultivation of grape varieties European grapes face significant challenges as salinity reduces their ability to adapt to local conditions, necessitating the adoption of precise research methodologies to control external conditions and study their response under controlled laboratory conditions. The study aimed to investigate the effect of Melatonin and Meta-topolin on plant hormones, proline content, and enzyme activity in Crimson seedless grapevine branches grown under salt stress in vitro.

2-Materials and Methods: The study was conducted in the Plant Tissue Culture Laboratory / Department of Horticulture and Landscape Architecture / College of Agriculture / Al-Qasim Green University during the research season (2024-2025), to conduct tissue culture experiments, using plant branches of the Crimson seedless grape variety grown in vitro under salt stress conditions. The first experiment included three different concentrations of NaCl (0, 75, 150) mmol. L^{-1}, and the second experiment included three concentrations of growth regulators (Melatonin, Meta-topolin) (0.4 and 0.8 MEL, Metato 1) mg. L^{-1}. MS medium, abbreviated as Murashige and Skoog salts (1962), was used for cultivation, and was prepared by withdrawing the required quantities prepared previously from basic solutions. All solutions were stored at 4°C in constant darkness, and a mass of 4.43 g L^{-1} was used according to the recommended instructions. 30% dietary sucrose, 100 mg L^{-1} myo-inositol, and 0.1 mg L^{-1} NAA were added, as well as 7 g L^{-1} agar to solidify the medium. The pH of the medium was adjusted to 5.7 ± 0.1 using a few drops of HCl or 0.1 N NaOH. The medium was placed on a hot plate magnetic stirrer to dissolve the agar and homogenize the MS medium components, and was immediately dispensed into pre-sterilized culture tubes at a rate of 10 ml per tube. The tubes containing the culture medium were then transferred to an autoclave for sterilization at 121°C and 1.04 kg/cm2 for 20 min. The tubes were then removed and allowed to cool and solidify at room temperature, making them ready for culture. All tools used in culture, including forceps, blade holders, and beakers, were sterilized in an autoclave at 121°C and 1.04 kg/cm2 for 20 min (Salman, 1988). After being removed, the forceps and blade holders were subjected to dry heat sterilization in a standard gravity convection oven at 50°C (±1°C) for 24 continuous hours. They were then transferred to a stratified air sterilization cabinet, sterilized with 99% alcohol, and then flame-blasted to remove the alcohol. Tissue cultures for grapes were established according to Al-Dahimi (2009), and included several stages, including:

2-1 The emergence stage: Fresh branches of 10-20 cm in length were collected from 10-year-old Crimson seedless grape vines growing in the field, from the horticulture unit of Al-Mahawil Station, one of the facilities of the General Company for Horticulture and Forestry - Ministry of Agriculture. In the preparation room, all leaves and thorns were manually removed and washed with a surface detergent solution (0.1% Tween® 20). They were then surgically sectioned using a sterile scalpel into longitudinal sections of 2.5-2 cm in length, ensuring that each section contained a single node explant. They were then washed with tap water (25±2°C) for 30 minutes to

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remove residual contaminants. They were transferred to the cultivation cabin for surface sterilization, where they were sterilized using 70% ethyl alcohol for 10 seconds, then immersed in a Clorax solution containing 5% (NaOCl) at a concentration of 20% for 20 minutes (Nayyef et al., 2022), with continuous shaking to remove air bubbles formed on the plant parts. They were then rinsed with sterile distilled water for three consecutive rinses for 5 minutes each, to remove contamination. Surface and harmful to the sterilizing material, and to maintain the viability of the plant parts. Then, the sterilized plant parts were transferred to sterile Petri dishes, and their ends in contact with the disinfectant were cut to a length of about 1 cm using sterile microdissecting forceps and a sterile standard scalpel blade, thus transforming them into a suitable state for culture. Each node was planted individually in a test tube containing 10 ml of MS medium. The tissue cultures were incubated in an environmentally controlled growth chamber for 4 weeks at a temperature of 25 ± 2°C and a light intensity of 1000 lux (55 ± 5 µmol $m^{-2} s^{-1}$) with a photoperiod of 16 h light/8 h dark (Smith, 2013).

2-2 Multiplication Stage: To multiply the cultures obtained from the emergence stage to obtain large numbers of shoots for the study, the resulting shoots were cut and cultured in MS medium supplemented with BA: 2.0 mg. L^{-1} (9.1 µM) + NAA: 0.1 mg. L^{-1} (0.54 µM), Singh et al., (2000b). Tissue cultures were incubated in an environmentally controlled growth chamber for four weeks under the same conditions as the cultures in the emergence stage.

2-3 Rooting Stage: To root the cultures obtained from the multiplication stage to obtain rooted plantlets, 3 cm long shoots were taken and cultured in MS medium supplemented with 0.5 mg. L-1 IBA and tissue cultures were incubated in an environmentally controlled growth chamber for four weeks at 25 ± 2°C and light intensity of 1000 lux (55 ± 5 µmol $m^{-2} s^{-1}$) with 16 h light/8 h dark photoperiods. The shoots started to grow, develop and root.

2-4 Conducting the experiment: after obtaining a contamination-free tissue culture and producing sterile plantlets of the grape variety used in the study for use in subsequent experiments. A factorial experiment was carried out with ten replications for each treatment to study the effect of sodium chloride concentrations and the interaction with growth regulators (Melatonin, Meta-topolin) on the phytohormone content, enzyme activity and proline content in grape shoots in vitro. By cultivating the plants in MS medium supplemented with 30 $g.L^{-1}$ sucrose and 2 mmol of BA with 7 $g.L^{-1}$ agar, NaCl was added to the medium at three levels, namely (150, 75, 0) $mmol.L^{-1}$, and the amount of NaCl added to the medium was 3.383 g/L for the concentration of 75 $mmol.L^{-1}$ and 8.766 g/L for the concentration of 150 $mmol.L^{-1}$, and two concentrations of the growth regulator Melatonin were added, 0.4 and 0.8 $mg.L^{-1}$, and one concentration of the growth regulator Meta-topolin, 1 $mg.L^{-1}$. The stock solution was prepared by dissolving 25 mg of powder (Melatonin or Meta-topolin) in 25 ml of distilled water and placing it in a laboratory mixer to ensure its dissolution and homogeneity, as well as for the control treatment. Each treatment concentration was considered as a separate treatment and were as follows: 0.0 mg L^{-1} Control, 0.4 mg L^{-1} Melatonin, 0.8 mg L^{-1} Melatonin, 1 mg L^{-1} Meta-topolin, 0.4 mg L^{-1} Melatonin + 1 mg L^{-1} Meta-topolin, 0.8 mg L^{-1} Melatonin + 1 mg L^{-1} Meta-topolin. Then, the pH of the medium was adjusted with a digital pH meter calibrated with standard solutions to 5.7 ± 0.1 by adding standard acidity buffers (0.1 M) drop by drop to achieve the desired acid or alkaline gradient, with continuous stirring. The plants were cultured in glass jars containing 50 ml of previously prepared solid MS medium, with ten replicates for each treatment. They were incubated in an environmentally controlled growth chamber for 6 weeks at 25 ±

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2°C and light intensity of 1000 lux (55 ± 5 µmol m^{-2} s^{-1}) with a light period of 16 h light/8 h dark. At the end of the incubation period, the following measurements were taken:

Catalase activity (CAT) was determined (µmol/g fresh weight/min) : using a spectrophotometer according to the method followed by (Aebi et al., 1984). This method is based on the amount of change in absorbance at a wavelength of 240 nm.

Peroxidase activity (POD) was determined (absorbance unit/g fresh weight) : according to the method followed by (Kim et al., 1988) using a spectrophotometer. This method is based on the amount of change in absorbance at a wavelength of 420 nm. The activity of the POD enzyme was calculated using the equation (Lateef et al., 2021).

Estimation of proline acid (µg. g^{-1} fresh weight) : The amount of proline amino acid in the branches grown in media prepared with NaCl was estimated according to the method of Bates et al. (1973) using a spectrophotometer. This method uses the amount of change in absorbance at a wavelength of 520 nm. The experiment was implemented according to the CRD design with a two-factor factorial layout with ten repetitions for each treatment.

3-Results and Discussion: Estimation of the plant hormone auxin Table (1) indicators revealed significant differences between NaCl concentrations in the auxin content in the branches of the plants, as the treatment without adding NaCl outperformed by giving the highest content rate of 151.12 µg/kg fresh weight, compared to the 150 mmol. L^{-1} treatment, which gave the lowest content rate of 113.77 µg/kg fresh weight. It is noted from the table that the auxin content of the branches of the plants, after six weeks of cultivation in MS media supported by specific concentrations of growth regulators (Melatonin, Meta-Topolin) increased significantly, as the interaction treatment 0.8M + Mt1 mg outperformed. L^{-1} gave the highest content rate of 149.6 µg/kg fresh weight, compared to the control treatment in the absence of growth regulators, which recorded the lowest content rate of 121.33 µg/kg fresh weight. The interaction of NaCl with the growth regulators Melatonin and Meta-Topolin had a significant effect on the auxin content of the branches, as the interaction treatment of 0 mmol. L^{-1} of NaCl with the addition of growth regulators 0.8M + Mt1 mg. L^{-1} gave the highest content rate of 171.10 µg/kg fresh weight, compared to the interaction treatments of 150 mmol. L^{-1} of NaCl with no addition of growth regulators and 150 mmol. L^{-1} of NaCl with the addition of 0.4 M, 0.8 M growth regulators gave the lowest content rates of (103.80, 104.70, 107.50) µg/kg fresh weight.

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Table 1: Effect of Melatonin, Meta-Topolin and their interactions on the content of the plant hormone auxin in the branches of Crimson seedless grape variety grown under salt stress conditions in vitro.

Concentration and type of growth regulator mg/L^{-1} (T)	NaCl concentration mmol.L^{-1} (S)			Growth regulator concentration rate
	0	75	150	
0	131.50	128.70	103.80	121.33
0.4 M	136.00	132.50	104.70	124.40
0.8 M	142.60	136.90	107.50	129.00
1 Mt	160.10	141.10	120.30	140.50
0.4 M + 1 Mt	165.40	148.90	121.80	145.37
0.8 M + 1 Mt	171.10	153.20	124.50	149.6
معدل تركيز NaCl	151.12	140.22	113.77	
LSD (0.05)	4.54 = S*T 1.85 = S 2.62 =T			

Cytokinin estimation Table (2) indicators revealed significant differences between NaCl concentrations in cytokinin content in the plantation branches, as the treatment without NaCl addition was superior by giving the highest content rate of 89.33 µg/kg fresh weight, compared to the 150 mmol. L^{-1} treatment which gave the lowest content rate of 71.88 µg/kg fresh weight. It is noted from the table that the cytokinin content of the plantation branches, after six weeks of cultivation in MS media supported by specific concentrations of growth regulators (Melatonin, Meta-Topolin) increased significantly, as the interaction treatment 0.8M + Mt1 mg was superior. L^{-1} gave the highest content rate of 106.87 µg/kg fresh weight, compared to the control treatment in the absence of growth regulators, which recorded the lowest content rate of 51.13 µg/kg fresh weight. The interaction of NaCl with growth regulators Melatonin and Meta-Topolin had a significant effect on the cytokinin content of branches, as the interaction treatment of 0 mmol. L^{-1} NaCl with the addition of growth regulators 0.8M + Mt1 mg. L^{-1} gave the highest content rate of 114.50 µg/kg fresh weight, compared to the interaction treatment of 150 mmol. L^{-1} of NaCl without adding growth regulators, which gave the lowest content rate of 47.50 µg/kg fresh weight.

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Table 2: Effect of Melatonin, Meta-Topolin and their interactions on the content of the plant hormone cytokinin in the branches of the Crimson seedless grape variety grown under salt stress conditions in vitro.

Concentration and type of growth regulator mg/L^{-1} (T)	NaCl concentration mmol.L^{-1} (S)			Growth regulator concentration rate
	0	75	150	
0	52.10	53.80	47.50	51.13
0.4 M	82.40	66.40	58.70	69.17
0.8 M	86.40	73.60	63.50	74.50
1 Mt	90.10	78.40	69.66	79.39
0.4 M + 1 Mt	110.50	103.60	93.20	102.43
0.8 M + 1 Mt	114.50	107.40	98.70	106.87
معدل تركيز NaCl	89.33	80.53	71.88	
LSD (0.05)	2.772 = S*T		1.132 = S	1.600 =T

Estimation of Catalase Enzyme (CAT) Activity The indicators in Table (3) revealed a significant effect of NaCl on the activity of the catalase enzyme in the plant branches, as the 150 mmol. L^{-1} NaCl treatment was superior by giving the highest activity of the catalase enzyme, which reached 5.029 µmol/g fresh weight/min, compared to the 0 NaCl treatment, which gave the lowest activity, which reached 2.372 µmol/g fresh weight/min. It is noted from the table that the activity of the catalase enzyme in the plant branches, after six weeks of cultivation in MS media supported by specific concentrations of growth regulators (Melatonin, Meta-Topolin), increased significantly, as the interaction treatment 0.8M + Mt1 mg was superior. L^{-1} gave the highest catalase activity of 4.393 µmol/g fresh weight/min, compared to the control treatment in the absence of growth regulators, which recorded the lowest activity of 2.917 µmol/g fresh weight/min. The interaction of NaCl with growth regulators Melatonin and Meta-Topolin had a significant effect on the catalase activity in the plant branches, as the interaction treatment of 150 mmol. L^{-1} of NaCl with the addition of growth regulators 0.8M + Mt1 mg L^{-1} gave the highest activity of 6.150 µmol/g fresh weight/min, compared to the control treatment 0 NaCl without adding growth regulators, which gave the lowest activity of 1.750 µmol/g fresh weight/min.

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Table 3: Effect of Melatonin, Meta-Topolin and their interactions on catalase activity in Crimson seedless grapevine branches grown under salt stress conditions in vitro.

Concentration and type of growth regulator mg/L^{-1} (T)	NaCl concentration mmol.L^{-1} (S)			Growth regulator concentration rate
	0	75	150	
0	1.750	2.890	4.110	2.917
0.4 M	2.090	3.080	4.670	3.280
0.8 M	2.170	3.670	4.990	3.610
1 Mt	2.450	3.880	5.165	3.832
0.4 M + 1 Mt	2.890	3.970	5.090	3.983
0.8 M + 1 Mt	2.880	4.150	6.150	4.393
معدل تركيز NaCl	2.372	3.607	5.029	
LSD (0.05)	0.159 = S*T		0.065 = S	0.092 =T

Estimation of the activity of the enzyme (POD) Peroxidase The indicators in Table (4) revealed a significant effect of NaCl salt on the activity of the peroxidase enzyme, as the 150 mmol. L^{-1} NaCl treatment was superior by giving the highest activity of 67.29 absorption units/g fresh weight, compared to the 0 NaCl treatment, which recorded the lowest activity of 28.68 absorption units/g fresh weight. It is noted from the table that the activity of the peroxidase enzyme in the branches of the plants, after 6 weeks of cultivation in MS media supported by specific concentrations of growth regulators (Melatonin, Meta-Topolin), increased significantly, as the interaction treatment 0.8M + Mt1 mg. L^{-1} was superior by giving the highest activity of 55.74 absorption units/g fresh weight, compared to the control treatment in the absence of growth regulators, which recorded the lowest activity of 39.86 absorption units/g fresh weight. The interaction of NaCl with growth regulators Melatonin and Meta-Topolin had a significant effect on the activity of peroxidase enzyme in the branches of the plants, as the interaction treatment of 150 mmol. L^{-1} of NaCl with the addition of growth regulators 0.8M + Mt1 mg L^{-1} gave the highest activity of 80.12 absorption units/g fresh weight, compared to the control treatment of 0 NaCl without adding growth regulators, which gave the lowest activity of 22.80 absorption units/g fresh weight.

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Table 4: Effect of Melatonin, Meta-Topolin and their interactions on the activity of the enzyme (POD) Peroxidase in the branches of Crimson seedless grapes grown under salt stress conditions in vitro.

Concentration and type of growth regulator mg/L^{-1} (T)	NaCl concentration mmol.L^{-1} (S)			Growth regulator concentration rate
	0	75	150	
0	22.80	38.77	58.00	39.86
0.4 M	25.14	39.29	61.18	41.87
0.8 M	28.07	44.12	64.10	45.43
1 Mt	30.18	45.14	67.87	47.73
0.4 M + 1 Mt	31.00	52.16	72.44	51.87
0.8 M + 1 Mt	34.88	52.22	80.12	55.74
معدل تركيز NaCl	28.68	45.28	67.29	
LSD (0.05)		2.523 = S*T	1.030 = S	1.456 =T

Proline content in branches Table (5) indicators revealed a significant effect of NaCl salt on the proline content of the branches of the plants, as the 150 mmol. L^{-1} NaCl treatment outperformed by giving the highest content rate of 49.82 µg. g^{-1} fresh weight, compared to the 0 NaCl treatment which recorded the lowest content rate of 38.79 µg. g^{-1} fresh weight. It is noted from the table that the proline content in the branches, after six weeks of cultivation in MS media supported by specific levels of growth regulators (Melatonin, Meta-Topolin), increased significantly, as the interaction treatment 0.8M + Mt1 mg. L^{-1} outperformed by giving the highest content rate of 47.24 µg. g^{-1} fresh weight, compared to the control treatment in the absence of growth regulators which recorded the lowest content rate at 41.12 µg. g^{-1} fresh weight. The interaction of NaCl with growth regulators Melatonin and Meta-Topolin had a significant effect on the proline amino acid content in the branches of the plants, as the interaction treatment of 150 mmol. L^{-1} of NaCl with the addition of growth regulators 0.8M + Mt1 mg. L^{-1} gave the highest content rate of 55.15 µg. g^{-1} fresh weight compared to the control treatment of 0 NaCl without adding growth regulators, which recorded the lowest content rate of 37.23 µg. g^{-1} fresh weight.

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Table 5: Effect of Melatonin, Meta-Topolin and their interactions on the proline amino acid content in the branches of Crimson seedless grapes grown under salt stress conditions in vitro.

Concentration and type of growth regulator mg/L^{-1} (T)	NaCl concentration mmol.L^{-1} (S)			Growth regulator concentration rate
	0	75	150	
0	37.23	40.06	46.08	41.12
0.4 M	38.07	44.06	47.02	43.05
0.8 M	38.58	44.26	48.73	43.86
1 Mt	39.19	44.80	50.17	44.72
0.4 M + 1 Mt	39.66	45.37	51.75	45.59
0.8 M + 1 Mt	40.03	46.55	55.15	47.24
معدل تركيز NaCl	38.79	44.18	49.82	
LSD (0.05)		2.23 = S*T	0.91= S	1.29 =T

Tables (1, 2, 3, 4, 5) show a significant decline in the auxin and cytokinin contents of plant branches growing under salt stress conditions in vitro. This can be explained by previous studies that indicated that plant hormone concentrations are affected in plants exposed to salt stress, leading to a systematic imbalance in hormonal signaling networks, manifested in a significant decrease in the concentrations of growth-stimulating hormones, compared to a significant increase in the levels of growth inhibitors. Research by Jing et al. (2023) showed a sharp decline in the levels of endogenous auxin (IAA) in multiple horticultural and field plants under stress conditions, attributed to a disruption in protein metabolism. This disruption, in turn, affects amino acid levels, particularly tryptophan, which is the main substrate for auxin biosynthesis (IAA) within the plant cell, causing a disturbance in the concentration of endogenous auxin. These results are generally consistent with the findings of a study (Vila et al., 2024) that exposed 11 acidic origins to salt stress in vitro, using different concentrations of NaCl in nutritional media. To analyze the regeneration process in vitro, they showed an increase in enzymatic degradation and biosynthesis, due to the increased activity of the cytokinin oxidase (CKX) enzyme responsible for degrading cytokinins into inactive forms (Werner et al., 2010). According to the results of our study, exposure to NaCl significantly increased the content of catalase and peroxidase enzyme activity in the branches, and the content of the amino acid proline. The reason for the increase in catalase activity may be attributed to the fact that it protects cells from secondary oxidative stress resulting from salinity. This enzyme is also considered a first line of defense against oxidative toxicity, as it is an effective biomarker for the presence of hydrogen peroxide and oxygen radicals ($H_2 O_2$) emitted from plastids and mitochondria of plants exposed to various stresses (De la et al., 2024). This enzyme is also characterized by its good ability to decompose ($H_2 O_2$) and convert it into water and oxygen, which promotes the restoration of metabolic balance (Al-Hattab, (2018). The increase in peroxidase (POD) activity with increasing NaCl concentration may be attributed to the excessive generation of reactive oxygen

species (ROS), resulting from salt stress, which causes oxidation of structural proteins and membrane lipids, leading to molecular damage to the structure of nucleic acids. In addition, as a defensive response, the plant stimulates antioxidant enzymes, including peroxidase (Faize et al., 2010). Peroxidase works to mitigate the harmful effects of salt stress by catalytically decomposing reactive oxygen species (ROS) (Kawano, 2003). The increase in proline, one of the most sensitive amino acids as a biomarker of stress severity, may be attributed to its increased levels and accumulation in the studied plant as an osmoprotective effect. It contributes to intracellular osmotic adjustment and detoxification of reactive oxygen species (ROS), while protecting the membrane structure without harming cellular metabolism. It also plays a crucial role in osmoregulation (Sharma et al., 2019). The relatively high enzyme activity of peroxidase with increasing Melatonin concentration may be due to Melatonin enhancing plant resistance to salt stress via a two-way mechanism: a direct mechanism by immediately scavenging reactive oxygen species (ROS), and an indirect mechanism by enhancing antioxidant activity. Furthermore, exogenously added Melatonin reduces ROS levels (Li et al., 2019). Moreover, meta-tubulin enhances antioxidant defense mechanisms by raising the levels of peroxidase and catalase enzymes and activating the formation of glutathione, which reduces oxidative stress caused by salinity (Syeed et al., 2021). Regarding proline, the significant increase observed with Melatonin treatment is attributed to the fact that stress causes the accumulation of free radicals (ROS) within plant cells. Melatonin has a physiological role in combating oxidative stress by stimulating the synthesis of enzymes that remove reactive oxygen species, such as peroxidase and catalase, and non-enzymatic ones, such as glutathione (Liang et al., 2019). The results of this study are consistent with those of Maola et al., 2023. Conclusion: The results of the study showed that adding different concentrations of NaCl to the nutrient medium had an adverse effect on the content of plant hormones (auxin, cytokinin) in the branches, while it led to an increase in the activity of antioxidant enzymes (catalysts, peroxidases) and the content of the amino acid proline in the branches. Therefore, these indicators constitute an integrated diagnostic system for determining the tolerance of the studied European grape variety (Crimson seedless) to salt stress using plantlets in in vitro tests. Growth regulators (Melatonin, Meta-Topolin) stimulated an increase in the tolerance of the plants to salt stress. The interaction treatment of 0.8M + Mt1 mg. L^{-1} outperformed during the study, giving the highest and best results, followed by the interaction treatment of 0.4M + Mt1 mg. L^{-1}. Therefore, it is recommended to adopt the cultivation of the studied grape variety Crimson seedless as a suitable alternative in soils suffering from salinity. Evaluation of the comprehensive feasibility of employing plant growth regulators (Melatonin, Meta-Topolin) on grape plants to improve their ability to tolerate NaCl salt in saline soils when desired to be planted there, and their adoption in micropropagation processes, due to their ability to enhance the tolerance of grape plants to salt stress in in vitro.

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