Effect of growth regulators on vegetative parameters of (Catharanthus roseus L.) shoots grown under salt stress conditions in vitro

Fatima Saad Atiyah Mohammed J. H. Al-shareefi Ban Mohammed Hussain Ali Altaei
2E-mail: shareefi@agre.uoqasim.edu.iq 3E-mail: ban.moh@agre.uoqasim.edu.iq
3-1Department of Horticulture, College of Agriculture, University of Al-Qasim Green, Iraq.

Abstract :

This study was conducted in 2023–2024 at the Agriculture Faculty of Al-Qasim Green College in the Plant Tissue Culture Laboratory of the Department of Horticulture. To investigate the effect of growth regulators and sodium chloride on the vegetative indicators of vinca rose in vitro, the experiment included two factors, the first factor Nacl at four concentrations (0, 50, 100, 150) mmol.L-1 and the second factor three types of growth regulators (putrescine , melatonin and brassinolide) at a concentration of 0.5 mg.L-1 , in addition to the comparison treatment. As a factorial experiment with two factors, ten repetitions, and a completely randomized design (CRD). The average number of shoots, percent dry weight, and chlorophyll content of the shoots were all outperformed by the sodium chloride concentration of 0 mmol.L-1, whereas the average length of the shoots was outperformed by the concentration of 50 mmol.L-1. While the results showed a significant excelled of the growth regulators, the treatment with 0.5 mg.L-1 melatonin gave the haste average of the number and length of shoots, the percentage of dry weight, and the chlorophyll content of the shoots. The interaction between the experimental factors also had a significant effect on the indicators studied, as the interaction treatment of 50 NaCl and 0.5 melatonin was superior in the average number and length of shoots, and the treatment of 0 NaCl and 0.5 melatonin was superior in the percentage of dry weight and chlorophyll content of shoots.

Keywords: plant tissue culture , Melatonin, Putrescine, Brassinolide, stress.

Introduction:

Periwinkle Madagascar, scientific name Vinca rosea (Catharanthus roseus L.) is one of the most important medicinal plants of the Apocynaceae family, which includes a large group of genera reaching approximately 411 genera and 4650 species (1). It is an evergreen herbaceous plant that is widely cultivated in warm regions of the world, and is grown as an ornamental plant in gardens and parks due to the cultivar of its flower colors and the long flowering period (2). This plant occupies a prominent position in the list of medicinal plants, as it contains more than 130 alkaloids, especially terpene alkaloids and their derivatives. Among these important compounds are Vincristine and Vinblastine, which are used in the treatment of cancer(3) .The problem of salinity is one of the most important problems facing the agricultural sector in different regions of the world, especially arid and semi-arid regions, as salt stress affects the activity of enzymes, the ability of photosynthesis, and also leads to an increase in osmotic potential, ion toxicity, and the generation of reactive oxygen species (ROS) in plant cells, which can cause oxidative damage at high concentrations (4). The use of tissue culture technology in

ISSN 2072-3857

studying the effect of salt stress on plants, as these technologies provide a homogeneous growth medium in terms of salt content and environmental conditions, as such studies may be difficult under field conditions due to their overlap with other different stresses related to soil and climate, and the overlap of such factors with salinity leads to difficulty in conducting the testing and evaluation process and understanding the mechanism of tolerance. This technology is also considered an effective means in producing many medical drugs, as it helps in the rapid production of these materials with high concentrations and purity and under sterile conditions free of diseases, in addition to continuous production throughout the year without being restricted to the planting season (6):(5) .Due to the wide spread of the phenomenon of soil and irrigation water salinity that limits the expansion of plant cultivation, it was found necessary to use means to reduce the severity of the harmful effects of salinity, so research has turned to the use of many growth regulators such as hormones, vitamins, and some organic substances. that help increase the growth rate and production in such conditions for their effective role in overcoming the harmful effect of salt stress by causing the accumulation of food transformation products or working as antioxidants or increasing the content of carotene and glutathione and some antioxidant enzymes such as SOD, CAT that work to protect plant cells from active oxygen compounds that cause stress (7). Researchers have recently focused on the evidently beneficial effects of brassinolide, putrescine, and most recently, melatonin. These substances are crucial for morphological phenomena and for controlling internal biochemical responses. Putrescine is Polyamines amine-containing, low molecular weight,They have been considered as a new kind of plant biostimulant, which play vital roles in diverse plant growth and developmental processes, and environmental stress responses (8).Brassinosteroids (BRs) are a new kind of phytohormones that play an essential role in plant development. Intensive research conducted on BRs reveals that they elicit a broad spectrum of physiological and morphological responses in plants, including stem elongation, leaf bending and epinasty, induction of ethylene biosynthesis and proton pump activation, synthesis of nucleic acid and proteins, regulation of carbohydrate assimilation and allocation, and activation of photosynthesis. Furthermore, BRs can protect plants from various biotic and abiotic stresses, such as those caused by salt, high temperatures, and heavy metals (9).The melatonin is a hormone involved in regulating plant growth, aerial organ development, root morphology, and the floral transition. The universal antioxidant activity of melatonin and its role in preserving chlorophyll might explain its anti-senescence capacity in aging leaves. Recent evidence also indicated that melatonin functions in the plant's response to stress(10). These substances are crucial for morphological phenomena and for controlling internal biochemical responses.Therefore, the research aimed to know the effect of growth regulators on the vegetative indicators of vinca rose under salt stress conditions 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 NaCL and growth regulators on the biochemical compounds of vinca rose in vitro. The study

ISSN 2072-3857

was carried out according to a CRD design with two factors and ten replicates. The first factor included four concentrations of NaCL (0, 50, 100, 150) mmol.L-1, and the second factor, three types of growth regulators (Putrescine, melatonin and brassinolide) used at a concentration of 0.5 mg.L-1 , in addition to control . Explant, represented by the shoots and nodes of a vinca rosea plant with a length of 5-10 cm, were taken from one-year-old seedlings growing in plastic pots and transported to the laboratory all leaves were removed from them and placed under running water and liquid soap for 30 minutes to get rid of the dirt and dust stuck to them. After that, then transferred the explant to the Laminar Air Flow Cabinet to perform the sterilization process and they were immersed in solution containing 1% sodium hypochlorate for 20 minutes, then washed with distilled water three times. Use the MS medium with the addition of sucrose 30 gm.L-1 and agar 7 g/L BA 1.5 mg.L-1. Then medium is then placed on the thermal magnetic mixing device to dissolve the agar and homogenize the nutrient medium. Then distribute the medium directly into the planting bottles at approximately 30 ml and sterilize in an autoclave at a temperature of 121 °C and a pressure of 1.04 kg.cm2 for 20 minutes. Then, the explants were cut to a length of about 1 cm containing a single node, and they were cultured on the MS medium inside the laminar airflow cabinet. After that, the shoots were incubated at temperatures of 25 ± 1 for 21 days. Then the shoots resulting from the initiation stage were then cut to a length of 1 cm with a single node, and they were cultivated on the same MS medium to which the NaCL and growth regulators were added. For four weeks, the seedlings were kept in the growth chamber at a temperature of 25 ± 1 and 16 hours of light and 8 hours of darkness. To study the effect of growth regulators and sodium chloride on the average number of shoots and length of shoots and the percentage of dry matter in the shoots and the content of chlorophyll in the shoots.

Results and discussion- :

Average number of shoots: The results in Table (1) show significant differences between the Nacl concentrations in the average number of shoots, as the concentration (0.0 mmol.L-1) reached the highest average number of shoots (3,890) compared to the concentration (150 mmol.L-1), which had the lowest average. The same table also shows that there is a significant effect between the concentrations of the growth regulators on the average number of shoots, as the concentration (0.5 mg.L-1) of melatonin gave the highest rate of (3,698) shoots, compared to the treatment without addition, which had the lowest rate. In the interaction treatment with 50 NaCl and 0.5 melatonin, the number of shoots was the highest, reaching (4.590) shoots, compared with treatment 150 mmol.L-1 of NaCl and 0 mg.L-1 of growth regulators which recorded the lowest rate.

ISSN 2072-3857

Table (1) Effect of growth regulators on the average number of vinca rosea shoots cultivated in vitro under salt stress conditions following a four-week culture

Average NaCl	growth regulators mg.L^{-1} (T)				NaCl mmol.L^{-1} S)(
	Brassinolide 0.5	Melatonin 0.5	Putrescine 0.5	Control 0.0	
3.8900	4.407	3.970	3.887	3.297	0
3.458	2.453	4.590	3.387	3.400	50
3.163	3.527	3.200	2.24	3.683	100
2.684	2.933	3.030	2.573	2.200	150
	3.33	**3.698**	**3.022**	**3.145**	**Effect of growth regulators**
S: 0.2282	**T: 0.2282**		**S*T :0.4565:**		**L.S. D**

Shoot length rate: The results in Table (2) show significant differences between NaCl concentrations in shoot length rate. The concentration (50 mmol.L-1) gave the highest shoot length rate of (4.885) cm, compared to the concentration (150 mmol.L-1) which had the lowest rate. The same table also shows that there is a significant effect between the concentrations of growth regulators on shoot length rate. The concentration (0.5 mg.L-1) of melatonin gave the highest rate of (5.107) cm, compared to the treatment without addition, which showed the lowest rate. The interaction treatment with 50 NaCl and 0.5 melatonin measured the highest rate of shoot length of (5.750) cm, compared with treatment 150 mmol.L-1 of NaCl and 0 mg.L-1 of growth regulators which recorded the lowest rate

.

Table (2) Effect of growth regulators on the shoot length of vinca rosea cultivated in vitro under salt stress conditions following a four-week culture

Average NaCl	growth regulators mg.L^{-1} (T)				NaCl mmol.L^{-1} (S)
	Brassinolide 0.5	Melatonin 0.5	Putrescine 0.5	Control 0.0	
4.703	4.743	5.437	4.307	4.327	0
4.885	4.487	5.750	4.887	4.417	50
4.254	4.603	4.790	3.940	3.683	100
4.058	4.333	4.453	4.073	3.373	150
	4.542	**5.107**	**4.302**	**3.950**	**Effect of growth regulators**
S :0.3017	**T : 0.3017**		**S*T: 0.6034**		**L.S. D**

Dry weight percentage in shoot: The results in Table (3) show significant differences between the Nacl concentrations in the percentage dry weight of the shoots. The concentration (0.0 mmol.L-1) gave the highest rate of (16.29)% compared to the concentration (150 mmol.L-1) which showed the lowest rate. The same

ISSN 2072-3857

table also shows that there is a significant effect between the concentrations of the growth regulators on the percentage dry weight of the shoots. The concentration (0.5 mg.L-1) of melatonin gave the highest rate of (16.73) %, compared to the treatment without addition, which showed the lowest rate. The interaction treatment with 0 sodium chloride and 0.5 melatonin recorded the highest percentage of dry weight of (17.91)%, compared with treatment 100 mmol.L-1 of NaCl and 0 mg.L-1 of growth regulators which recorded the lowest rate.

Table (3) Effect of growth regulators on the percentage of dry matter of vinca rosea cultivated in vitro under salt stress conditions following a four-week culture

Average NaCl	**growth regulators mg.L-1 (T)**				**NaCl mmol.L^{-1} (S)**
	Brassinolide 0.5	**Melatonin 0.5**	**Putrescine 0.5**	**Control 0.0**	
16.29	15.65	17.91	16.63	14.96	0
15.64	14.71	17.68	14.75	15.42	50
14.77	13.36	16.36	16.05	13.32	100
14.49	15.37	14.94	13.96	13.70	150
	14.77	**16.73**	**15.34**	**14.35**	**Effect of growth regulators**
S :1.619		**T :1.619**	**S*T: 3.239**		**L.S. D**

con

Chlorophyll content in shoot (mg. 100g-1 fresh weight): The results in Table (4) show significant differences between the NaCl concentrations in relation to the chlorophyll content of the shoots. The concentration (0.0 mmol.L-1) showed the highest value of (34.11) mg. 100g-1 fresh weight, compared to the concentration (100 mmol.L-1) which showed the lowest value. The same table also shows that there is a significant effect between the concentrations of the growth regulators on the chlorophyll content of the shoots. The concentration (0.5 mg.L-1) of melatonin gave the highest rate of (34.85) mg. 100g-1 fresh weight, compared to the treatment without addition, which had the lowest value. While the interaction treatment 0 NaCl and 0.5 melatonin recorded the highest rate of chlorophyll content in the shoots and (38.44) mg. 100g-1 fresh weight, compared with treatment 100 mmol.L-1 of NaCl and 0.5 mg.L-1 of putrescine which recorded the lowest rate .

Table (4) Effect of growth regulators on the chlorophyll content of vinca rosea cultivated in vitro under salt stress conditions following a four-week culture

Average NaCl	**growth regulators mg.L-1 (T)**				**NaCl mmol.L-1 S) (**
	Brassinolide 0.5	**Melatonin** 0.5	**Putrescine** 0.5	**Control 0.0**	
34.11	37.21	38.44	32.97	27.83	0
32.02	31.50	33.55	30.68	32.36	50
27.89	28.14	34.78	23.66	24.99	100
29.54	31.05	32.65	29.42	25.06	150

ISSN 2072-3857

	31.97	**34.85**	**29.18**	**27.56**	**Effect of growth regulators**
S :3.613		**T :3.613**	**S*T: 7.228**		**L.S. D**

Tables (1, 2, 3 and 4) show a significant decrease in the average number and length of shoots, percentage dry weight and chlorophyll content in the shoots of vinca rosea grown under salt stress conditions in vitro. The decrease in the number and length of shoots could be due to a disturbance of physiological processes and nutrient balance due to increased osmotic potential and decreased amount of water and nutrients entering the cells, as well as ionic toxicity due to the accumulation of some toxic ions such as chloride and sodium (7): (12). The decrease in dry weight, may be due to a reduction in the rate of photosynthesis and reduced uptake of essential nutrients, leading to a disruption of vital metabolic processes. Salt stress also plays a role in inhibiting the natural growth regulators in plant tissue, which negatively affects plant growth and reduces their biomass (8). The decrease in total chlorophyll content in the leaves, is due to the effects of salinity on the ionic balance in the plant, which negatively affects the uptake of chlorophyll components such as nitrogen, magnesium and iron. In addition, the accumulation of sodium and chloride ions inhibits the formation of various pigments or destroys the chloroplast membrane. (9) :(12). On the other hand, the results of the same tables show that growth regulators play an important role, as melatonin had a significant effect on the average number and length of shoots, the percentage of dry weight and the chlorophyll content in the shoots of vinca rose grown in vitro. This effect is due to the fact that melatonin, similar to IAA, promotes cell division and elongation and maintains the integrity of cell membranes and chlorophyll pigment (10): (13). Melatonin and auxin are involved in primary biosynthesis in plants, both from the same amino acid, and thus their role is similar to that of indole acetic acid. Melatonin also contributes to the regulation of plant hormone gene expression during growth and development processes (11): (14.(

References:

.1
Yang, L., Wen, K.-S., Ruan, X., Zhao, Y.-X., Wei, F., & Wang, Q. (2018). Response of plant secondary metabolites to environmental factors. Molecules, 23(4), 762.

.2 Dubey, A., Tiwari, D., Srivastava, K., Prakash, O., & Kushwaha, R. (2020). A discussion on vinca plant. Journal of Pharmacognosy andPhytochemistry, 9(5), 27–31.

.3 Das, S., & Sharangi, A. B. (2017). Madagascar periwinkle (Catharanthus roseus L.): Diverse medicinal and therapeutic benefits to humankind. Journal of Pharmacognosy and Phytochemistry, 6(5), 1695–1701.

.4 Hasanuzzaman, M., Bhuyan, M. H. M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. Al, Fujita, M., & Fotopoulos, V. (2020). Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants, 9(8), 681.

.5 Mulabagal, V., & Tsay, H.-S. (2004). Plant cell cultures-an alternative and efficient

source for the production of biologically important secondary metabolites. International Journal of Applied Science and Engineering, 2(1), 29–48.

.6 Georgiev, M. I., Weber, J., & Maciuk, A. (2009). Bioprocessing of plant cell cultures for mass production of targeted compounds. Applied Microbiology and Biotechnology, 83, 809–823.

,

.7 Fariduddin, Q., Zaid, A., & Mohammad, F. (2019). Plant Growth Regulators and Salt Stress: Mechanism of Tolerance Trade-Off BT - Salt Stress, Microbes, and Plant Interactions: Causes and Solution: Volume 1 (M. S. Akhtar (ed.); pp. 91–111). Springer Singapore. https://doi.org/10.1007/978-981-13-8801-9_4.

.8 Mustafavi, S. H., Naghdi Badi, H., Sękara, A., Mehrafarin, A., Janda