Impact of the growth regulator B.A in salt stress tolerance of Swingle Citrumelo Citrus rootstock shoots' in vitro

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Abstract

The study was carried out in 2023–2024 in the tissue culture lab of Al-Qasim Green University's College of Agriculture. to determine how well tissue-cultured Swingle Citrumelo citrus rootstock shoots can tolerance salt stress while being influenced by the growth regulator B.A. The experiment included two factors; the first factor was three concentrations of the growth regulator B.A (2, 0, 3) mg.L-1 . and the second factor was four concentrations of NaCl (0, 50, 100, 150) mmol. L-1. The results showed a significant decrease in growth indicators (number of shoots, branch length, percentage of dry weight), while the results showed a significant increase in sodium percentage and proline content when increasing sodium chloride concentrations. While the addition of the growth regulator BA to the nutrient medium recorded a significant increase in growth indicators (number of shoots, branch length, percentage of dry weight) and a decrease in sodium percentage and proline content in the shoots.

Keywords: B.A, salt stress, Citrumelo , in vitro.

***Research paper from MSc thesis for the first author.**

Introduction

Citrus belongs to the Rosacea family and the Citrus genus, which is considered one of the most important genera from an economic point of view. Citrus commercial varieties are propagated by grafting them onto appropriate rootstocks that are created using seeds or tissue culture methods. Given that the tree depends in its basic structure on the rootstock, which represents its root system, and the scion, which represents the vegetative system, and the existence of a physiological relationship between them in terms of the effect of the rootstock on several horticultural characteristics of the grafted variety, including the shape, size, and growth of trees, the age of fruiting, production, and tolerance to environmental factors, as well as tolerance to widespread diseases, the interest in improving trees should focus on improving both the rootstock and the scion equally [13]. Citrus cultivation in Iraq faces many problems, especially in the central and southern regions, due to salinity, drought, and high groundwater levels. Salinity is one of the main soil problems that limits plant growth and productivity in many regions of the world, especially dry and semi-dry regions, as it causes a significant decrease in the growth and yield of plants growing in these soils through an imbalance in nutritional and hormonal balance and the toxic effect of ions [5]. The use of in vitro cultivation techniques in studying the mechanics and physiology of tolerance to salinity, as these techniques provide a homogeneous growth medium in terms of salt content and environmental conditions, is such that these studies may be difficult under field conditions due to their interference with other different stresses

related to soil and climate, and the interference of such factors with salinity leads to difficulty in conducting the testing and evaluation process and understanding the tolerance mechanism. Many systems have been used to evaluate the tolerance of citrus fruits to salt stress, and among these systems is the use of the ex vivo plant tissue culture technique by cultivating buds and shoots multiplied in agricultural media with different levels of NaCl salt. The cultivation of shoots is one of the good systems used in testing plant tolerance to salinity [7]. Due to the widespread nature of the phenomenon of soil and irrigation water salinity, which limits the expansion of plant cultivation in Iraq, it was found necessary to use means to reduce the severity of the harmful effects of salinity. Recent research has tended to use growth regulators that play an important role in morphological phenomena and their regulation of biochemical reactions internally. In this study, we decided to identify their effects on regulating plant growth in reducing the effect of salinity on the Swingle Citrumelo Citrus rootstock growing in vitro living.

Material and Methods

The experiment was conducted in the Plant Tissue Culture Laboratory, College of Agriculture, Al-Qasim Green University, during the year 2024 to evaluate the tolerance of tissue-cultured Swingle Citrumelo rootstock shoots to salt stress under the influence of the growth regulator BA. The experiment was carried out according to the CRD design with two factors and ten replicates. The first factor included three concentrations of BA (0, 2, 3 mg) and the second factor included four concentrations of sodium chloride (0, 50, 100, 150 mmol/L).In this experiment, young shoots of Swingle Citrumelo rootstock with a length of 5-10 cm from 1-2-year-old seedlings were used. In the preparation room of the tissue culture laboratory, leaves and thorns were removed and cut to a length of 2 cm so that each part contained one node. They were then washed with running water for 30 minutes, after which they were transferred to the stratified air cabin for sterilization. They were sterilized with 70% alcohol for 30 seconds and then sterilized with sodium hypochlorite solution (1%) with the addition of 3 drops of Tween 20. After the sterilization process was complete, they were washed with sterile distilled water three times to remove the effect of the sterilizing material inside the culture cabin.

In this experiment, the ready-made MS culture medium was used, with sucrose added to it at an amount of 3% and agar at an amount of 7 g. L-1, and myo-inositol at an amount of 100 mg. L-1. The medium was then placed on a magnetic thermal mixing device to dissolve the agar and homogenize the culture medium. Then, the medium was distributed directly into the culture glassware 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.

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washed with sterile distilled water three times to remove the effect of the sterilizing material inside the culture cabin [14.[

The sterilized plant parts were transferred and placed in sterile dishes inside the laminar, and their ends that were in contact with the sterile material were cut so that the length of the node became about 1 cm. The single nodes were planted on the special culture medium for the emergence of the plants using sterile tissue culture tools. Then, the plants were incubated at a temperature of 23-25°C and a light intensity of 1000 lux for 16 hours of light and 8 hours of darkness. After five weeks, the plants resulting from the emergence stage were cut into stem nodes, and each node was planted in glass tubes containing 10 ml of MS medium supplemented with 1 mg/L of BA and 0.1 mg/L of NAA. The process was repeated several times until reaching the required number of plants needed for the study. Then, the plants were transferred to a culture medium free of growth regulators for two weeks to remove the effect of growth regulators.

The vegetative parts were cut into cuttings, each cutting containing 2 nodes, and planted in glass tubes containing 10 ml per tube of the same MS medium used in the branch formation stage, in addition to four concentrations of sodium chloride (NaCl), which are (., 50, 100, 150) mmol.L-1, and three concentrations of the growth regulator BA, which are (., 2, 3) mg.L-1, in addition to 10 replicates for each treatment. The plants were incubated in the growth chamber at a temperature of 23-25°C and a lighting intensity of 1000 lux for 16 hours and for four weeks. At the end of the incubation period, the studied indicators were taken.

Results and Discussion

-1Average number of shoots

The results shown in Table (1) showed significant differences in the number of shoots for sodium chloride salt, as the treatment without adding sodium chloride recorded the highest rate of (4.588), which did not differ significantly with the treatment of adding sodium chloride 50 mmol.L-1, which recorded (3.992), while they differed significantly with the treatments of adding sodium chloride 150 and 100 mmol.L-1, which gave the lowest rate in the number of shoots, which reached (2.874 and 2.873), respectively. It is noted from the table that there is a significant effect on the rate of the number of shoots growing in media equipped with different concentrations of the growth regulator BA, as the concentration of 2 mg/L recorded the highest rate in the number of shoots, which reached 4.303, which did not differ significantly with the concentration of 3 mg/L, which recorded 4.171, compared to the comparison, which recorded the lowest rate, which reached 2.272. The table also showed that the interaction between sodium chloride salt and the growth regulator benzyl adenine (BA) had a significant effect on the average number of shoots, as the interaction treatment (0 mmol. L-1 sodium chloride and 2 mg. L-1 of BA) gave the highest average number of shoots, reaching (5.678), compared to the interaction treatment (100 mmol. L-1 sodium chloride without adding the growth regulator BA), which gave the lowest average number of shoots, reaching (1.866.(

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Table (1) Effect of benzyl adenine (BA) on the average number of shoots of Swingle Citrumelo rootstock grown under salt stress conditions in vitro.

Sodium chloride) NaCL(Mmol.L^{-1} (S)	benzyl adenine (BA) mg.L^{-1} (T)			Effect of sodium chloride
	0	2	3	
NaCL: 0	2.513	5.678	5.574	4.588
NaCL: 50	2.571	4.827	4.578	3.992
NaCL: 100	1.866	3.477	3.275	2.873
NaCL: 150	2.138	3.228	3.256	2.874
Effect of benzyl adenine	2.272	4.303	4.171	
L.S.D	S : 0.4620	T : 0.4001	S*T : 0.8002	

-2Average of shoots length (cm(

The results in Table (2) showed significant differences between the concentrations of sodium chloride salt in the average branch length, as the treatment of adding 50 mmol.L-1 recorded the highest average branch length (3.592) cm, which did not differ significantly from the control treatment that recorded (3.468) cm, compared to the treatment of adding 150 mmol.L-1, which recorded the lowest average (2.850) cm. It is noted from the table that there is a significant effect on the average length of the shoots growing in media equipped with different concentrations of the growth regulator BA, as the concentration of 3 mg.L-1 recorded the highest average (3.642) cm, compared to the control treatment that recorded the lowest average branch length (2.272) cm. The interaction between sodium chloride concentrations and growth regulator (BA) had a significant effect on the branch length rate, as the interaction treatment (0 mmol/L sodium chloride and 3 mg/L of BA) gave the highest branch length rate (4.053) compared to the interaction treatment (150 mmol/L sodium chloride without adding the growth regulator BA), which gave the lowest branch length rate (2.323.(

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Table (2) Effect of benzyl adenine (BA) on the branch length rate of Swingle Citrumelo rootstock grown under salt stress conditions in vitro

Sodium chloride) NaCL(Mmol.L^{-1} (S)	benzyl adenine (BA) mg.L^{-1} (T)			Effect of sodium chloride
	0	2	3	
NaCL: 0	2.713	3.637	4.053	3.468
NaCL: 50	3.490	3.483	3.803	3.592
NaCL: 100	2.340	3.203	3.433	2.992
NaCL: 150	2.323	2.950	3.277	2.850
Effect of benzyl adenine	2.717	3.318	3.642	
L.S.D	S : 0.3515	T : 0.3044	S*T : 0.6089	

-3Dry weight percentage

The results of Table (3) show significant differences in the percentage of dry matter for sodium chloride salt, as the treatment without adding sodium chloride recorded the highest rate of 16.21% compared to the treatment adding sodium chloride 150 mmol/L, which gave the lowest rate in the percentage of dry matter of 13.39%. The same table shows a significant effect in the percentage of dry matter in the shoots growing in media equipped with different concentrations of the growth regulator BA, as the concentration of 2 mg/L recorded the highest percentage of dry matter (15.21)%, which did not differ significantly from the concentration of 3 mg/L, which recorded (14.51)%, compared to the concentration of 0 mg/L, which recorded the lowest percentage of dry matter in the shoots (14.34)%. The interaction between sodium chloride and the growth regulator benzyl adenine (BA) had a significant effect on the percentage of dry matter, as the interaction treatment (0 mmol. L-1 sodium chloride and 2 mg. L-1 of BA) gave the highest percentage (16.88)%, compared to the interaction treatment (150 mmol. L-1 sodium chloride without adding the growth regulator BA), which gave the lowest percentage of dry matter in the shoots (12.89.(

Table (3) Effect of benzyl adenine (BA) on the percentage of dry weight in Swingle Citrumelo rootstock shoots grown under salt stress conditions in vitro.

Sodium chloride) NaCL(Mmol.L^{-1} (S)	benzyl adenine (BA) mg.L^{-1} (T)			Effect of sodium chloride
	0	2	3	
NaCL: 0	15.34	16.88	16.42	16.21
NaCL: 50	14.72	15.11	14.88	14.90
NaCL: 100	14.42	14.97	13.38	14.26
NaCL: 150	12.89	13.90	13.38	13.39
Effect of benzyl adenine	14.34	15.21	14.51	
L.S.D	S : 0.978	T : 0.847	S*T : 1.694	

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-4The percentage of sodium in the shoots

The results of Table (4) show significant differences in the percentage of sodium for sodium chloride salt, as the treatment without adding sodium chloride recorded the lowest percentage (3.602) compared to the treatment adding sodium chloride 100 and 150 mmol.L-1, which gave the highest percentage of sodium in the shoots (5.119, 4.985) %. The same table shows a significant effect on the percentage of sodium in the growing shoots in media equipped with different concentrations of the growth regulator BA, as the concentration of 2 mg.L-1 recorded the lowest percentage of sodium in the shoots (4.342), which did not differ significantly with the concentration of 3 mg.L-1, which recorded (4.437) % compared to the comparison treatment, which recorded the highest percentage (4.728) %. The interaction between sodium chloride and the growth regulator benzyl adenine (BA) had a significant effect on the percentage of sodium, as the interaction treatment (0 mmol. L-1 sodium chloride and 2 mg. L-1 of BA) gave the lowest percentage (3.301)% compared to the interaction treatment (100 mmol. L-1 sodium chloride without adding the growth regulator BA), which gave the highest percentage (5.763)%. Regarding the interaction between growth regulator and salt, there is no difference between treatments (0, 2) and (0, 3.(

Table (4) Effect of benzyl adenine (BA) on the percentage of sodium in the shoots of the Swingle Citrumelo rootstock grown under salt stress conditions in vitro.

Sodium chloride) NaCL(Mmol.L^{-1} (S)	benzyl adenine (BA) mg.L^{-1} (T)			Effect of sodium chloride
	0	2	3	
NaCL: 0	3.796	3.301	3.708	3.602
NaCL: 50	4.4780	4.220	4.210	4.303
NaCL: 100	5.763	4.489	5.104	5.119
NaCL: 150	4.875	5.356	4.724	4.985
Effect of benzyl adenine	4.728	4.342	4.437	
L.S.D	S : 0.2654	T : 0.2299	S*T : 0.4597	

-5Proline content in shoots

The results of Table (5) showed significant differences between the concentrations of sodium chloride salt in the proline content, as the treatment adding 150 mmol.L-1 recorded the highest proline content (0.656) compared to the control treatment, which recorded the lowest content (0.410). It is noted from the table that there is a significant effect on the proline content in the shoots growing in media equipped with different concentrations of the growth regulator BA, as the control treatment recorded the highest effectiveness in the proline content (0.566) compared to the concentration of 3 mg.L-1, which recorded the lowest effectiveness in the proline content in the shoots (0.489). The interaction between

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the concentrations of sodium chloride and the growth regulator benzyl adenine (BA) had a significant effect on the proline content, as the interaction treatment (150 mmol. L-1 sodium chloride without adding the nucleation regulator BA) gave the highest proline content (0.738) compared to the interaction treatment (0 mmol. L-1 sodium chloride with the addition of 3 mg. L-1 of BA), which gave the lowest proline content in the shoots (0.385(

Table (5) Effect of benzyl adenine (BA) on the proline content of Swingle Citrus rootstocks grown under salt stress conditions in vitro .

Sodium chloride) NaCL(Mmol.L^{-1} (S)	benzyl adenine (BA) mg.L^{-1} (T)			Effect of sodium chloride
	0	2	3	
NaCL: 0	0.420	0.426	0.385	0.410
NaCL: 50	0.443	0.415	0.464	0.441
NaCL: 100	0.662	0.575	0.503	0.580
NaCL: 150	0.738	0.626	0.605	0.656
Effect of benzyl adenine	0.566	0.511	0.489	
L.S.D	S : 0.0727	T : 0.0630	S*T : 0.1259	

The results in Tables (1, 2, 3) indicate a significant decrease in the average number and length of shoots, the percentage of dry weight, and shoots of the Citrus rootstock Swingle Citrumelo grown in vitro under salt stress conditions. The decrease in the number and length of shoots when the concentration of sodium chloride salt increases is attributed to

toxic ions as a result of the increase in salt concentration negatively affects and changes the growth of the plant, the distribution of nutrients, and the instability of the cell membrane, which results from the replacement of calcium with sodium, and thus causes a decrease in the efficiency of carbon metabolism [10, 15]. As for the decrease in dry weight, it may be due to the lack of absorption of important nutrients and thus the imbalance in vital metabolic processes. The decrease in dry weight is associated with a decrease in the rate of plant elongation and the number of buds, as the dry matter is the net production of the photosynthesis process and the effect of salinity on the growth and development of shoots through its effect on the hormonal and ionic balance and vital processes, as well as its effect on the amount of water absorbed to the extent that it hinders the occurrence of cell division and elongation [12, 6]. In addition, the ionic toxicity resulting from the accumulation of some depends on the balance between photosynthesis and respiration [8,4.[

The reason for the increase in the percentage of sodium in the citrus rootstock shoots may be due to the increase in its concentration in the nutritional medium, which leads to an increase in the absorption of Na+ ions, as sodium works to withdraw calcium from the membranes to replace it, which increases the damage in them, and the membranes change from selectivity to complete permeability, which makes the entry of sodium faster and thus its accumulation in the cytoplasm and cell vacuoles, in addition to the competition between sodium and potassium for absorption

sites [1,9]. The reason for the accumulation of the amino acid proline in the tissues of plants exposed to salt stress is due to the fact that proline is a defensive method followed by the plant when exposed to salt stress, as high salinity concentrations cause a disturbance in the growth of the plant, so the plant resorts to forming free amino acids such as proline to reduce the toxic effect of their accumulation inside the cells, and the speed of its construction increases the speed of its accumulation in addition to inhibiting the effectiveness of the enzymes that oxidize proline [11, 3, 2.[

References

[1]Alhasnawi, A. N., Che Mohd Zain, C. R., Kadhimi, A. A., Isahak, A., Mohamad, A., & Wan Yusoff, W. M. (2017). Accumulation of antioxidants in rice callus ('Oryza sativa'L.) induced by beta-glucan and salt stress. Australian Journal of Crop Science, 11(1), 118-125.

[2] AL-Taey, D. K. A., S. S. M. AL-Azawi, M. J. H. AL-Shareefi, and A. R. AL-Tawaha. (2018) . Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (Lactuca sativa var. Parris Island) Res. Crops 19 : 441-449. DOI : 10.31830/2348- 7542.2018.0001.14

[3] Ashraf, M. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experiment Botany, 59(2) : 206-216.

[4] Davidson, H. R., & Campbell, C. A. (1984). Growth rates, harvest index and moisture use of Manitou spring wheat as influenced by nitrogen, temperature and moisture. Canadian journal of plant science, 64(4), 825-839.

[5] Flowers, T.J. (2004). Improving crop salt tolerance. Journal of Experimental Botany, 55(396) : 307-319.

[6] Ghaleb., W. Sh. ; Sawwan, J.S. ; Akash, M.W. and Al-Abdallat, A.M. (2010). In vitro response of two Citrus rootstocks to salt stress. International Journal of Fruit Science ,10(1):40-53.

[7] Hu, C.Y. and Wang ,P.J. .(1983). Meristem, shoot tip and bud cultures. In : D.A. Evans ; W.R. Sharp; P.V. Ammirato and Y. Yamada (eds.) Handbook of Cell Culture .(pp.177-227.(

[8] Maola, M., Ayied, S., & Jaber, M. (2023, April). The Role of Sodium Chloride, Putrescine, and Melatonin on The Chemical Markers of Green Beans. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 4, p. 042037). IOP Publishing.

[9]Maola, M., Jaber, M., & Ayied, S. (2023, April). The Impact of Salinity, Putrescin, and Melatonin Interactions on Green Bean Vegetative Indicators. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 4, p. 042036.(

[10]Mohammed , A.S.; A.P. Mohammed .; M.B. Rashid.; A. Rashid. A.Tahira and .A. Naheed. 2011. Salt stress effects on some morphological and physiological characteristics of okra (Abelmoschus esculentus L.) . Soil Environ. 30(1): 66-73.

[11]Muslim, D. A., Al-Shareefi, M. J., & Alazawi, S. S. M. (2023, April). Effect of Salicylic Acid and Nano-Silicon of the Enzymatic Activity for Potato Shoots Grown Under Salt Stress in Vitro. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 10, p. 102006.(

[12]Muslim, D. A., Al-Shareefi, M. J., & Mahmoud, S. S. (2023). Effect of Salicylic Acid and Nano Silicon on the Morphological Traits of Potato Shoot Grown in Vitro Under Salt Stress. In IOP Conference Series: Earth and Environmental Science (Vol. 1158, No. 10, p. 102005.(

[13]Nayyef , M. Noaman .2013. Evaluation of Some Citrus Rootstocks for Water Stress In Vitro. PhD thesis. Department of Horticulture ,Faculty of Agriculture University of Kufa Iraq.

[14]Nayyef, M. N., Awadh, H. A. A., Kadhim, Z. K., AL-Shareefi, M. J., & Abdulhussein, M. A. A. 2022. A comparative study between the effect of benzyl adenine and adenine sulphate on growth and multiplication of banana shoots (Musa spp.) IN VITRO. Int. J. Agricult. Stat. Sci. Vol, 18(1), 385-390.

[15]Taiz, L. and Zeiger, E. (2006).Plant Physiology. The Benjamin/Cummings Publishing Company, Inc. Redwood City, California, USA. P.559.

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