Effect of Salt Stress in Callus of Catharanthus roseus

Safa Hussein Abbas Fadhel1* Omar H. Obaid2*
2Department of Plant Production Techniques, Al-Musayyib Technical College, Al-Furat Al-Awsat Technical University, Babylon, Iraq.
Author email: 1safa.hussain.tcm.51@student.atu.edu.iq
2omarobaid46@gmail.com

Abstract
This experiment was conducted in the plant tissue culture laboratory of the Department of Plant Production Technologies, Al-Musayyib Technical College, Al-Furat Al-Awsat Technical University to study the effect of salt stress, NaCl, on the callus of the Catharanthus roseus plant. The study included two experiments. The first experiment is hypochlorite sterilization with three concentrations (1.5, 2, 3) % for different periods of (10, 15, 20) minutes. The second experiment is to study the effect of salt stress (NaCl) at three concentrations (50,100,150) mmol on the alkaloids vincristine and vinblastine, and the callus content of Na, Cl Ions, and Proline. In addition to dry weight and Fresh weight of callus, an MS medium containing 1mg.L-1 2,4-D and BA 0.5 mg.L-1. The results showed the contamination percentage decreased to 0.00% at the concentration of 3% within 20 minutes. There were significant differences in the concentrations of the alkaloids Vincristine and Vinblastine when plants were exposed to salt stress. The concentration is 50 mmol gave the highest concentration of Vincristine, which reached 19.243, and Vinblastine which reached 25.653. While the 50 mmol of Nacl showed significant superiority in callus content Na, Cl Ions, and Proline. In addition, there were significant differences in the fresh and dry weight of callus when exposed to salt stress, where control treatment was excelled by giving the highest fresh weight, reaching 0.3100 mg. Regarding the dry weight, the control treatment excelled by giving it the highest dry weight, amounting to 0.0500 mg.

Keywords: Catharanthus roseus, callus, salt stress, Vincristine, Vinblastine, hypochlorate.

Introduction

Catharanthus roseus is considered a member of the Apocynaceae family, it is one of the evergreen ornamental plants that is distinguished by its medicinal importance. The Catharanthus roseus plant is widely grown as an ornamental plant in the central and southern of Iraq. Currently, interest in that plant has increased as it is considered an important plant to serve as a call to return to nature after knowing the danger of side effects of manufactured medicines and the fact that herbal medicine is the foundation on which pharmacological medicine is built [1.[

The origin of the genus name is Greek, consisting of two syllables: (katharose), which means pure, and (anthos), which means flower, referring to the softness and beauty of its flowers. Catharanthus roseus is considered a medicinal plant because it contains more than 100 monoterpenoid indole alkaloids and dimeric terpene alkaloids. Indole alkaloid is found in various plant organs. The stem and leaves also contain Bisindole Alkaloid (Vinblastine and Vincristine) [2] In the field of diseases, natural products, especially plant products, have been used to treat many of

ISSN 2072-3857

them for thousands of years in Mesopotamia, Egypt, China, India and the Greeks. As this is the first record of the use of medicinal plants by the Sumerians and Akkadians [3[

Alkaloids of Catharanthus roseus are considered among the most important anti-cancer alkaloids, but the quantities produced in the plant are very small and economically unfeasible. In addition to the high cost of their production, researchers prompted to use tissue culture technology to increase the plant content of these compounds, especially since the amount of these compounds is very affected by external factors and the environmental conditions that surround the plants, including biological factors [4]. The composition of the nutrient medium supplied with sodium chloride has effects on cell growth and the accumulation of secondary metabolites [5]. Sodium chloride is a salt that increases the negativity of the osmotic potential of the cell, which plays role in increasing the process of photosynthesis and thus increasing the production of secondary metabolites. The process of extracting both Vinblastine (VLB) and Vincristine (VCR) is usually expensive and need extra efforts, which has prompted researchers to work on extracting these medically valuable compounds in larger quantities through modern biotechnology [4]. Based on the above, this study aims to examine the effect of salt stress on the concentration of the alkaloids vincristine and vinblastine in callus. In addition to study the effect of salt stress on the fresh and dry weight of callus.

Materials and methods

This experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Plant Production Technologies, Al-Mussaib Technical College, Al-Furat Al-Awsat Technical University to study the effect of NaCl salt stress on the callus of the Catharanthus roseus plant. This study included two experiments:

Sterilization experiment, where used is hypochlorate with three concentrations (1.5, 2, 3) % for a different time period of (10, 15, 20) minutes, The percentage of contamination was calculated in this experiment.

Salt stress experiment, which prepared 1 molar of sodium chloride solution, 58.44gm of sodium chloride is dissolved in one liter of distilled water so that the solution becomes 1 molar, diluted to diluted concentrations of up to (0,50,100,150) mmol.L-1 according to [2]. In this experiment vincristine and vinblastine alkaloids were calculated by using HPLC device, and the concentrations injected into HPLC were prepared according to [6], and the Na mg.kg-1[7], Cl mg.kg-1 [8], proline mg.100g [9] content and fresh and dry weight of callus were also exanimated.

The experiment was designed according to a completely randomized design (C.R.D.), with ten replications, and its significance was tested according to the L.S.D test at a level of 0.05 using the GENSTAT program .

Results and discussion

-1The effect of percentage concentrations of sodium hypochlorite and the sterilization period on the percentage of contaminated plants week after culture.

Table (1) shows the effect of sodium hypochlorite concentrations and their interaction with sterilization periods on the percentage reduction in contamination rates in Catharanthus roseus plant. We notice from the table that the 2% concentration gave the lowest contamination percentage, which

ISSN 2072-3857

amounted to 6.6, and thus it significantly outperformed all other concentrations. The duration of sterilization had a significant effect on reducing the percentage of contamination, and the lowest value reached 26 % when the time period was 20 minutes. As for the interaction between concentrations and time, the highest percentage of contamination was at the first concentration (1.5%), with the first time (10) minutes, reaching 100%, and the lowest percentage of contamination at the third concentration (3%), with the third time (20) minutes, reaching 0.00%. Sterilization in the laboratory is an essential step for plant tissue culture, and the final results of culture in the laboratory depend directly on the efficiency of sterilization [10] The reason for using sodium hypochlorite in surface sterilization of plant parts may be due to its efficiency and its lack of harm to the explant at the appropriate concentration, concentration and time period. It is effective in reducing surface contamination of cultivated plant parts, and a concentration higher than the ideal concentration and a long period of time may lead to the death of plant parts, and this is consistent with what researchers [11] found. [12] who stated that high concentrations lead to the death of plant parts.

Table (1): The effect of percentage concentrations of sodium hypochlorite (NaOCL) and the sterilization period per minute on the percentage of contaminated plants for different periods after one week of culture.

Concentration (%)	Time/minute			concentration average
	10	15	20	
1.5	100.00	100.00	69.00	89.6
2	41.00	11.00	9.00	20.3
3	11.00	9.00	0.00	6.6
Time average	50.6	40	26	38.8
L.S.D 0.05	concentration 0.809	Time 0.809	Interaction 1.401	

-2

The effect of different concentrations of sodium chloride on the concentration of alkaloids after 45 days of culture

Table (2) shows the effect of salinity on the concentration of (vincristine, vinblastine). After HPLC analysis of the callus of Catharanthus roseus plant, the concentrations

ISSN 2072-3857

were all superior to control treatment that gave the lowest concentration vincristine 10.0 mg.L-1 The highest concentration of vincristine was recorded at 19.243 mg.L-1 in a medium supplemented with 50 mmol of NaCl, while the highest concentration of vinblastine was 25.653 mg.L-1 in a medium supplemented with 50 mmol of NaCl. These results are consistent with what studies have reported, in that an increase in the production and accumulation of secondary compounds and alkaloids, one of which is due to exposure of plants or their cells to salt stress, has been found in many studies in many plants, including Datura plants [13] Catharanthus roseus plants [14][15] [16.[

The content of alkaloids in the branches of Catharanthus roseus plant also increased when the plant was exposed to concentrations of 50 and 100 mmol of NaCl, in agreement with a previous study [17]. The increase in alkaloid compounds in the presence of salts perhaps due to the interference or influence of increasing the content of these cells of some alkaloid precursor compounds that contribute to increasing the biosynthesis of these compounds. This is done as means of countering excess salt ions, such as the increase in amino acids and organic acids that serve as storage for nitrogen that contributes to synthesis. Biosynthesis of alkaloid compounds under salt stress conditions [18 .[

There are significant differences between the treatments in the concentration of sodium ion Na+, as the 150 mmol treatment excelled on all treatments as it gave (2.816) mg.kg. This is a result of the accumulation of Na+ when salt concentrations increase in the cells, where the cells located at the base of the callus allow these ions to enter to increase the osmotic potential as a result of salt stress. That leads to the accumulation of these ions in the cytoplasm and vacuoles of cells exposed to salt stress, and this is consistent with [19] [20]. Regarding the chloride ion, significant differences appeared and the 50 mmol treatment was excelled and gave 19.6 mg.kg As for proline, the 50 mmol treatment excelled on the rest of the treatments, as it gave 13.647 mg.kg, as the increase in salt levels led to the accumulation of the amino acid proline in plant tissues and thus a disruption of osmotic activities inside the cell, where proline production increases in it as a result of its exposure to stress. In the cytoplasm of the cell and creating this state between the gap and the cytoplasm or acts as a protective factor for enzymes and cellular organelles in the cytoplasm.[21][22 [

ISSN 2072-3857

Table (2): The effect of different concentrations of sodium chloride added to (MS) medium with growth regulators 2,4-D 1 ml^{-1} and BA 0.5 ml^{-1} on the concentration of alkaloids and Na, Cl Ions and Proline after 45 days of culture

concentration NaCL Mmol. L^{-1}	Vincristine $mg.L^{-1}$	Vinblastine $mg.L^{-1}$	Na mg.kg	Cl mg.kg	Proline $mg.100gm^{-1}$
0	10.000	10.000	0.002	0.31	3.050
50	19.243	25.653	2.243	19.6	13.647
100	18.150	24.750	2.583	3.791	5.273
150	16.150	22.450	2.816	6.505	12.833
LSD0.05	0.2268	0.3416	0.0116	0.0941	0.05963

-3Effect of salt stress with different levels of salt on the fresh and dry weight of callus after 45 days.

Table (3) shows there were significant effect of sodium chloride salt concentrations on the fresh and dry weight of callus tissue. Control treatment outperformed the rest of the treatments and gave the fresh weight of 0.3100 mg, while the treatment gave 150 mmol.L- 1 the lowest fresh weight. It reached 0.1100 mg. The results also showed there were significant effect of sodium chloride salt concentrations on the dry weight of callus between the treatments. As control treatment outperformed the rest of the treatments and gave a dry weight of 0.0500, while the concentration of 150 mmol.L-1 gave the lowest dry weight of 0.0133. amalgam. In general, adding a stressful factor to the growth medium leads to inhibition of cell elongation and division, which negatively affects the growth rate of callus cells, as salt molecules work to hold water molecules, thus reducing the number of free water molecules available for absorption by callus cells, which negatively affects pressure. Fullness, or as a result of the ionic toxic effect of chlorine and sodium ions in the salt stress treatment [23]. The decrease in cell growth rates in callus exposed to salt stress may be due to a decrease in nutrients in the growth medium, as well as a decrease in the availability of nutrients necessary for plant growth [24] The differences in the response of plant callus to growth in salt media are due to the genetic nature of the plant in the nature of callus growth and its influence on levels of salt stress and the subsequent modifications in the shape of the callus cells that make them adapt to salt media and continue to grow at different rates depending on those modifications [25.[

ISSN 2072-3857

Table (3): Effect of salt stress with different levels of NaCl on the fresh and dry weight (mg) of callus after 45 days.

NaCl Mmol.L^{-1}	Fresh weight mg	dry weight mg
0	0.3100	0.0500
50	0.1700	0.0330
100	0.1533	0.0300
150	0.1100	0.0133
LSD0.05	0.05435	0.01441

Conclusions

Sterilization with sodium hypochlorite at a concentration of 3% for 20 minutes was effective in sterilizing the surface of plant parts, and we recommend using it on the leafy parts of other plants. The percentage of alkaloids changes with increasing stress, as shown by the results of the analysis of secondary compounds using an HPLC device for crushed callus with the combination (2,4-D mg.L-1 1 x 0.5 mg.L-1 BA) and sodium chloride salt added to the medium in different concentrations (0,50,100,150) mmol (50 mmol) gave the highest concentration of the active ingredients (vincristine and vinblastine). Also, there were changes with increasing stress as shown by the results of proline and Na and Cl Ions (50 mmol) gave the highest concentration of them. Adding salt stress represented by sodium chloride to the development medium led to changes in the fresh and dry weights, as the control treatment recorded the highest average for the fresh and dry weights.

ISSN 2072-3857

References

Al-Mamouri, A. A. K. 2020. The effect of biofertilization and NACL on the growth of Catharanthus roseus. Doctoral thesis. Musayyib Technical College. Al-Furat Al-Awsat Technical University. Ministry of Higher Education and Scientific Research. Iraq.

[2]Muhammad, B. A. and M. H. Mahdi. 2016. The effect of Phenylalanine on callus induction in Catharanthus roseus. Babylon University Journal of Pure and Applied Sciences.24(8.(

[3]Al-Adhari, M. A. H. and H. L. H. Al-Jubouri. 2016. Inducing the production of callus and the anti-cancer agent's vinblastine and vincristine using leaf tissue culture techniques of Catharanthus roseus using the fungi richoderma hahrzianum and fusarium oxysporum and using different techniques. College of Education for Girls. University of Kufa. Pp 98.

[4]Al-Memari, I. M. S; B. Z. Q. Bashi, and Al-Daoudi, I. J. 2014. The effect of adding 2,4-D, sodium chloride and vincristine on the callus content of Catharanthus roseus L. G.DON and the identification of some alkaloids using a high-performance chromatography device (HPLC). College of Agriculture and Forestry. University of Mosul. Ministry of Higher Education and Scientific Research. Iraq.

[5]Al-Amouri, Y. 2023. Study of morphological traits and genetic variations in callus cultures of Catharanthus roseus (Catharanthus roseus L.). Al-Baath University Journal - Agricultural Sciences and Biotechnology Series.45(2.(

[6]Gupta, M. M; D.V. Singh, A. K. Tripathi, R. Pandey, R. K. Verma, S. Singh, A. K. Shasany and Khanuja, S. P. S. 2005. Simultaneous Determination of Vincristine, Vinblastine, Catharanthine, and Vindoline in Leaves of Catharanthus roseus by High-Performance Liquid Chromatography. journal of Chromatographic Science.43. [1]

[7]CAES. 1999. Methods for the analysis of soil. Plant. Water. and Environmental samples, Agricultural and Environmental services Laboratories (CAES). University of Georgia.

[8]Gaines, T. P; M. B. Parker and Gascho, G. J. 1984. Automated determination of chlorides in soil and plant tissue by sodium nitrate. Agron. J. 76:371-374.

[9]Bates, L.S., R. Waldren, and I.D. Tear. (1973).Rapid determination of free proline of water-stress studies. Plant and Soil,9:205-207.

[10]Hesami, M; R. Naderi, and Tohidfar, M. 2019. Modeling and Optimizing in vitro Sterilization of Chrysanthemum via Multilayer Perceptron-Nondominated Sorting Genetic Algorithm-II (MLP-NSGAII). Front. Plant Sci., 10, 282.

[11]Haque, M. S; T. Wada and Hattori, K. 2003. Effect of Sucrose, Mannitol and KH2PO4 on proliferation of Root Tip Derived shoots and Subsequent Bulblet formation in Garlic. Asian Journal of Plant Sciences. 2 (12): 908 – 903.

[12]Elminadi, N. A. 2001. Studies on garlic production through tissue culture technique. M. Sc. Thesis. Agric, Cairo Univ.

[13]Al-Hatemi, K. T. K. 2006. A comparative study of the production of tropine alkaloids inside and outside the body in the two species of Datura metel Lin Datura innoxia mill. Doctoral dissertation. College of Science. University of Babylon.

ISSN 2072-3857

[14]Misra, N. and A. K. Gupta. 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in Cathatanthus roseus seedlings. J. Plant Physiol., 136:11-18.

[15]Zhao, J; W. H. Zhu, Q. Hu and Guo, Y.Q. 2000. Improvement of indole alkaloid production in Catharanthus roseus cell cultures by osmotic shock. Biotechnology Letters, 22(15): 1227-1231.

[16]Jaleel, C. A; B. Sankar, R. Sridharan, and Panneerselvam, R. 2008. Soil Salinity Alters Growth, Chlorophyll Content, and Secondary Metabolite Accumulation in Catharanthus roseus. Turk. J. Biol., 32: 79-83.

[17]Osman, M. E. H; S. S. Elfeky, K. Abo El-Soud and Hasan, A. M. 2007. Response of Catharanthus roseus shoots to salinity and drought in relation to Vincristine alkaloid content. Asian J. Plant Sci., 6(8) 1223-1228.

[18]Sato, F; Hashimoto, T. Hachiya, A. Tamura, K. I. Choi, K. B. Morishige, T. T. Morishige, H. Fujimoto, andYamada, Y. 2001. Metabolic engineering of plant alkaloid biosynthesis. Proceedings of the National Academy of Sciences, 98(1), 367-372.

[19]Liu, K.B. and S. X. Li. 1991. Effect of NaCl on element balance,peroxides isozyme and protein of lycopersicon esculantum leaf cultures and regenerated .Shoots .Scientia Hort,46:97-107.

[20]Cano, E. A; Perez-ALFocea, F. Moreno, V. Caro, M. and Bolarin, C. M. 1996. Responses to NaCl stress of cultivated and Wild tomato species and their hybrids in callus Cultures.Plant Cell Rep.,15:791-794.

[21]Van-Rensburg, L; Kruger, G. and Kruger, H. 1993. Proline accumulation as drought tolerance selection criterion its relationship to membrane integrity and chloroplast ultra structure in nicotine tobacco. L.J. plant physiol.,141:181-194.

[22]Delauney, A. J. and D.P. Verma. 1993. Proline biosynthesis and osmoregulation in plants. The plant .4(2):215-223.

[23]Bekheet, S. A. 2015. Effect of cryopreservation on salt and drought tolerance of date palm cultured in vitro. Sci Agric, 9 (3): 142-149.

[24]Rains, D. W; S. S. Croughan and T. P. Croughan. 1986. Isolation and characterization of mutant cell lines and plants: salt tolerance. In: Vasil, K. (ed.) Cell Culture and Somatic Cell Genetics of Plants.3. 537-547. Academic Press, Inc., NewYork.

[25]Hassan, N. S. and D. A. Wilkins. 1988. In vitro selection for salt tolerance lines in Lycopersicon peuvianum. Plant Cell Reports ,7:463.

ISSN 2072-3857