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Effect of different levels of NaCl on the growth and response of tomato (*Lycopersicon esculentum* Mill.) callus

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Abstract

This study attempted to figure out the impact of varying sodium chloride concentrations (0, 50, 100, 150, 200 mM) in the Murashige and Skoog nutritional medium on callus growth and the response of tomato plants. The findings indicated that low salt concentrations (50 mM) promoted callus growth relative to the control, whereas elevated salt concentrations resulted in a marked reduction in both the fresh and dry weight of the callus. The study found that proline content increased gradually with increasing salt stress, reflecting its role in enhancing cellular salt tolerance. Conversely, carbohydrate content showed a significant increase at higher concentrations, indicating a metabolic response associated with stress resistance. Electrolyte leakage levels gradually increased with rising salinity, -indicating damage to cell membrane integrity. These results confirm that salt stress inhibits cell growth and induces a range of physiological mechanisms aimed at mitigating the effects of high salinity.

Keywords: Salinity, tissue culture, callus, tomato, proline carbohydrate

Introduction

Salinity in soil and water is a major factor affecting tomato crop growth, yield, and quality. About 20% of all agricultural land and 33% of irrigated land worldwide face high salinity, leading to significant economic losses in tomato production (Inculet *et al*., 2019; Shrivastava & Kumar, 2015; A-lbaladejo *et al*., 2017) [12, 21, 2]. Excess salinity limits plant water uptake, resulting in osmotic stress (Munns and Tester, 2008) [16]. This results in ionic imbalance and oxidative damage. In order to adapt, plants initiate signaling pathways that regulate ionic equilibrium and osmotic adjustment (Zhang *et al*., 2014; Flowers *et al*., 2015) [27, 8].

Salinity has a direct impact on crop quantity and quality; therefore, productive traits must be considered when developing salt-tolerant varieties. However, these traits alone are insufficient, as salinity affects almost all physiological and biochemical aspects, making plant resilience enhancement require the integration of a large number of different physiological traits in breeding programs, including evaporation rates of the scion and rootstock (Singh *et al*., 1993), or from salt tolerance based genetic improvement (Cuartero *et al*., 2006) [7]. Tomato is moderately sensitive to the soil salinity and has been used in many of vitro experiments, research for salt tolerance and genetic studies because of its simple genome (2n=24) and genomic diversity characterization in the Solanaceae family (Bhatia *et al*., 2004) [4].

Producing of salt-tolerant plants could be envisaged, although the greatest problem is represented by screening thousands of plants as long as there are no reliable selection criteria for tolerant species. Thus, *in vitro* culture is a valuable approach not only for the production of salt-tolerant plants, but also for simultaneous rapid screening of genetic resources for salt tolerance stress. This *in vitro* approach makes it feasible to evaluate a considerable number of genotypes, since sterile-grown plants express their biomass-yielding salt stress resistance as determined by Tewary *et al*. (2000) [23].

Tissue culture approaches in plants have a long-term goal of enhancing crop improvement and the multiplication of new genotypes, as well supplementing conventional plant breeding. This method can be used to study plant physiology and genetics at the cellular level in a laboratory environment, and to improve genetic diversity in plants (Kacem *et al*., 2017) [13].

Plant tissue culture is a widely used technique that has gained attention in the recent years to improve genetic potential of tomato plants and produce salt-tolerant variety. Positive relationships of callus induction with general plant reaction to saline stress have been reported (Perez-Alfocea *et al*., 1994) [18].

These methods have been successful in the screening of stress-tolerant genetic materials under controlled conditions, and enabling the selection of tolerant lines that will be introduced from traditional breeding programs and transgenic transformations (Benderradji *et al*., 2012; Taratima *et al*., 2022) [3, 22].

Recently, studies have been revived on the evaluation of salt tolerance response in different crop species under *in vitro*

sterile growing condition. This approach has been applied to assess salt stress tolerance in crops like rice (Taratima *et al*.,2022) [22], wheat (Klay *et al*., 2024) [15], alfalfa (Yazıcılar & Bezirganoglu,2023) [26], Brassica species (Shahbazi *et al*.,2021) and eggplant Hannachi *et al*. 2021) [10].

In response to the universal issue of soil and water salinity globally, significant efforts have been directed towards comprehending the physiological factors associated with plant salinity tolerance, which serve as the foundation for plant breeders in creating more salt-resistant genetic variants.

This research aim to evaluate tomato plants' cellular-level response and tolerance to varying sodium chloride salt concentrations using plant tissue culture. Understanding these responses is crucial f-or selecting salt-stress-resistant cell lines.

Materials and Methods

The study carried out in the Tissue Culture Laboratory. Biology Department, College of Education, University of Basra, using the tomato (*Lycopersicon esculentum* Mill.) Super marimond cultivar approved for cultivation in Iraq.

Surface sterilization of seeds

The seeds were initially immersed in 70% ethyl alcohol and agitated gently for 5 minutes. Subsequently, they were rinsed with sterile distilled water. The seeds were superficially sterilized for thirty minutes with agitation in commercially available bleach (Clorox) solution, which contains 5% sodium hypochlorite, with the addition of one drop of Tween-20 surfactant per 100 ml. The seeds were ultimately rinsed three times with sterile distilled water. This process was conducted within a Laminar Air Flow Cabinet. The cabinet underwent pre-sterilization with 70% ethanol.

Preparing the nutrient medium for planting seeds and developing seedlings

Pre-sterilized seeds were cultured on regulator-free MS medium (Murashige &Skoog, 1962) [17] at four seeds per tube to obtain sterile seedlings for later use as explant sources.

Callus Induction

Sterile seedling cotyledons and true leaves were used for callus induction on the medium described in Table 1, and cultures were then incubated in darkness at 25±2 ºC in a growth room for four weeks.

-Table 1. Components of callus induction medium Concentration (mg/l)--Chemical--Full strengthBasal MS medium30000--Sucrose--170--Sodium dihydrogen orthophosphate--40--Adenine sulfate--0.5--Thiamine - Hcl--4--2,4-Dichlorophenoxy Acetic Acid(2,4-D)--22ip-) - Isopentenyle adenine-(-7000—Agar

Table 1. Components of callus induction medium

Chemical	Concentration (mg/l)
Basal MS medium	Full strength
Sucrose	30000
Sodium dihydrogen orthophosphate	170
Adenine sulfate	40
Thiamine – Hcl	0.5
2,4-Dichlorophenoxy Acetic Acid(2,4-D)	4
2ip (Isopentenyle adenine)	2
Agar	7000

Callus proliferation

After four weeks of callus formation Figure 1, both mass and size increased. A subculture was then established. The callus was divided into several sections. These sections were transferred to fresh nutrient media. The new medium had identical components to the callus induction medium, as shown in Table 1. For further proliferation, the cultures were incubated at 25±2'C in a growth room.

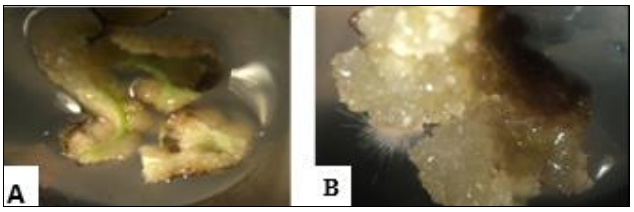

Fig 1: Callus tissue formed after four weeks of culture on the induction medium (A). Callus tissue proliferated after subculture on the induction medium for four weeks (B).

Salt stress induction

After obtaining a sufficient amount of callus, 150- mg of callus was taken and cultivated in culture media with components similar to the induction medium, with the addition of different concentrations of sodium chloride salt (0, 50, 100, 150, 200) mM to induce salt stress for four weeks Figure 2. The cultures were incubated in a growth chamber at 25'C, light 16 h, and darkness 8 hours. The following parameters were measured after four weeks of NaCl treatment of the callus:

1- Fresh and dry weight of callus

2- Measurement of proline content in callus

First, 0.5 g of fresh callus was taken and homogenized with 10 ml of 3% sulfosalicylic acid. The mixture was then filtered using Whatman's No. 2 filter paper. Subsequently, 2 ml of the filtrate was combined with 2 ml of ninhydrin and 2 ml of glacial acetic acid. The sample then incubated within water bath at 100 °C for one hour, subsequently cooled in an ice bath. Extraction was conducted using 4 ml of toluene until the chromatophore phase separated from the aqueous phase.

The intensity of the resulting color was determined by measuring visible light absorbance at 520 nm using a spectrophotometer, as method described by Bates *et al*., (1973).

3- Measurement of carbohydrate concentration in callus

About 0.5 g sample of fresh callus was homogenized with 2 ml of 1 N hydrochloric acid and heated in a boiling water bath for 20 minutes. The mixture was then cooled to room temperature and centrifuged at 2000 rpm for 5 minutes. Subsequently, 0.5 ml of the filtrate combined with 0.5 ml of solution of 5% phenol and 2.5 ml of concentrated sulfuric acid. Carbohydrate concentration was determined by measuring color intensity with a spectrophotometer at 490 nanometers, following the method described by Chitlaru & Pick (1989) [6].

4- Electrolyte leakage

Electrolyte leakage was estimated following Yadav *et al*. For each treatment, 100 mg of callus was washed with deionized distilled water and placed in test tubes with 10 ml

of distilled water. Samples were kept at 25'C for one hour. They were then stored 60 minutes to fully release electrolytes. After cooling to 25'C, the final electrical conductivity (L2) was measured. Electrolyte leakage was calculated using the following equation:

$$\text{Electrolyte leakage (EL)} = L1 / L2 * 100$$

5-Statistical analysis

The data were subjected to the analysis of variance (ANOVA), and the means were compared via the Least Significant Difference (L.S.D) test at level 0.05 using the GenStat program.

Results

Table 2 shows that increasing the sodium chloride concentration significantly affected the fresh weight of callus tissue. The 50 mM concentration recorded the highest average fresh weight, reaching 0.340 g, and this increase was significant compared to the- 0, 100, 150, and 200 mM concentrations. These concentrations did not show significant differences among themselves, as the fresh weight treatment, a fresh weight of 0.230- g was recorded. These results indicate that a moderate concentration of sodium chloride salt (50 mM) may stimulate cell growth of callus, while high levels of salinity caused a clear decrease in the fresh weight of the callus due to the negative effects of salt stress.

Table 2. Effect of different NaCl Concentrations on fresh weight of callus (g)

Concentrations of NaCl (mM)	0	50	100	150	200
Means	0.230	0.340	0.193	0.173	0.133
SD	0.05±	0.04±	0.070±	0.025±	0.045±
L.S.D at level 0.05	0.0884				

*SD / Standard Deviation L.S.D / Least Significant Difference

Table 3 results showed that sodium chloride treatment significantly affected dry weight. The concentration of 50 mM recorded the highest dry weight, reaching 0.032 g, and the difference was significant compared to the other treatments of 100, 150, and 200 mM. While the 200, 150, and 100 mM treatments did not show significant differences among each other, only the 150 and 100 mM treatments resulted in significantly decreased weight compared to the control treatment.

Table 3. Effect of different NaCl Concentrations on dry weight of callus(g)

Concentrations of NaCl (mM)	0	50	100	150	200
Means	0.022	0.032	0.019	0.016	0.014
SD	0.003±	0.0015±	0.005±	0.002±	0.003±
L.S.D at level 0.05	0.0061				

Table 4 shows that the proline content in callus tissue increased gradually and significantly with increasing sodium chloride concentrations in the culture medium. The highest proline without sodium chloride, at 0.130 mg/g, indicating salt stress. All treatments differed significantly, showing that proline content rises with salt stress.

Table 4. Effect of different NaCl Concentrations on proline content(mg/g)

Concentrations of NaCl (mM)	0	50	100	150	200
Means	0.130	0.309	0.551	0.774	1.069
SD	0.004±	0.012±	0.101±	0.048±	0.158±
L.S.D at level 0.05	0.1586				

Table 5 shows that increasing the NaCl concentration led to a gradual increase in the carbohydrate content of the callus. The carbohydrate content was lowest in the 0 mM control treatment, at 0.152 mg/g, and highest at 0.720 mg/g with the 200 mM concentration. The statistical analysis revealed significant differences between most treatments. Differences occurred between concentrations of 0, 100, 150, and 200 mM. However, the analysis did not show significant differences between 0 and 50 mM or between 150 and 200 mM.

Table 5. Effect of different NaCl Concentrations on carbohydrates(mg/g)

Concentrations of NaCl (mM)	0	50	100	150	200
Means	0.152	0.257	0.424	0.624	0.720
SD	0.03±	0.025±	0.150±	0.069±	0.103±
L.S.D at level 0.05	0.1621				

The results of the statistical analysis in Table 6 indicated that electrolyte leakage increased with rising NaCl concentration. The lowest rate was 12.07% in the control treatment. The highest rate reached 24.27% in the 200 mM treatment. Clear and significant differences appeared starting at 150 mM, where electrolyte leakage increased significantly. No significant differences were seen between the lower concentrations (0-100 mM). These results show that increased -salt stress weakens cell membrane integrity, causing higher ion leakage. High salinity levels damage membrane structure more than low salt treatments.

Table 6. Effect of different NaCl Concentrations on electroplate leakage (%)

Concentrations of NaCl (mM)	0	50	100	150	200
Means	12.07	14.23	16.83	21.00	24.27
SD	1.17±	1.28±	1.43±	1.71±	2.107±
L.S.D	2.874				

Discussion

The data revealed that the 50 mM concentration yielded the greatest values for both the fresh and dry weight of the callus in comparing with the other treatments. A further decline in fresh weight was noted, along with an elevation in the concentration of NaCl to 100, 150, and 200 mM.

The resulting response can be addressed within the established framework of salt stress effects on plants.

This is followed by the toxic effect of sodium (Na+, Cl-), which were responsible for disruption of vary metabolic processes beside increasing the production of free radicals and may damages cell membranes. This finding was aided by a number of studies showing that *in vitro* plant tissue culture is an effective tool for studying the effects of salinity on plant cells (Wijerathna *et al*., 2023) [25].

Regarding to the moderate concentration of sodium chloride salt at 50 mM, it had a boosting effect on callus growth, leading to a significant increase in both fresh and dry weight.

This effect may be attributed to the stimulation of vital processes necessary for growth and resistance to salt stress, including regulating the activity of certain enzymes, increasing the absorption of nutrients by cells in the growth medium, and maintaining turgor as a mechanism to resist salt stress. (Broadley & White, 2012; Geilfus, 2018; Tsunekawa *et al*., 2009) [5, 9, 24]. Our results align with Hongqiao *et al*., (2021) [11] who found that adding 5 mM sodium chloride to Arabidopsis growth medium increased plant biomass.

The findings of Rivera *et al*. (2022) [19] substantiate that a moderate concentration of 50 mM sodium chloride boosts the growth of tomato seedlings. As the NaCl levels in the growth medium reached 100 mM and beyond, the fresh weight diminished. This finding confirms the evidence presented by other researchers that they stated tissue exhibits heightened sensitivity to elevated salt concentrations. The detrimental consequences of salts encompass a reduction in growth rate and a diminished ability for water absorption.

The decrease in both fresh and dry weights with more sodium chloride may be due to cell division inhibition from increased osmotic pressure and negative ionic effects. High salt levels also reduce the ability to absorb water and nutrients. This is caused by lower water potential in the growth medium and ion toxicity from sodium ion accumulation within tissues (Sairam & Tyagi, 2004) [20]. These results agree with findings from many previous studies (Khuder& Al-Taei, 2015, Hannachi *et al*., 2021; Klay & Slim, 2024) [14, 10, 15].

Sodium chloride treatment at varying concentrations led to a significant increase in proline content, especially at higher levels. This proline accumulation is a key physiological strategy plants use to adapt to salt stress.

Also, Proline, a crucial amino acid in response to osmotic stress, functions as an osmoprotectant by preserving cellular osmotic pressure, stabilizing proteins and membranes, and aiding in the resistance to oxidative stress induced by reactive oxygen species (Verbruggen & Hermans, 2008; Szabados & Savouré, 2010) [31, 32]. Numerous studies indicate that proline accumulation markedly increases with exposure to elevated salt levels in both callus cultures and the entire plant.

For example, Sairam and Tyagi (2004) [20] argument that rising the levels of amino acid proline are correlated with improved cellular stability and tolerance for salinity and drought. Notably, these findings were further approved by numerous studies showing that proline accumulation in callus tissue serves as highly indicator of salt stress tolerance.

Also, this study argument that increasing the NaCl concentration gradually raised carbohydrate content in callus tissue. Significant differences appeared at 100 mM and above, but differences between 150 and 200 mM were not significant.

The response pattern found in this study were supported prior studies indicating that salt stress induces the accumulation of suitable solutes, including soluble sugars and starch, in numerous plants as an adaptive mechanism to salt stress. The effect of salinity on soluble sugar accumulation is linked to the plant's tolerance to salt stress (Abreu *et al*., 2013; Bezirganoglu, I., 2017) [1, 28]. The building up of compatible solutes during stress preserves ionic equilibrium, safeguards cellular integrity, and maintain osmotic balance via promoting continuous water uptake (Sairam *et al*., 2002) [29].

Elevated carbohydrate buildup in the callus tissues of various plant species which subjected to salt stress has been documented, including wheat callus (Khuder & A-l-Taei, 2015) [14] and tomato callus (Mohamed & Ismail, 2011) [30].

He conclusions of the present study indicated that electrolyte leakage (EL) dramatically escalated with rising NaCl concentration in the medium.

These results are consistent with what Zhou *et al*., (2024) [33] reported regarding electrolyte leakage as an indicator of cell membrane damage under salt stress. They demonstrated that the damage caused by salinity is due to relative dehydration and an increased intracellular Na+/Cl- content, leading to plasma membrane disruption and increased permeability.

Also, salt stress may lead to damage in cell plasma membranes due to overproduction of reactive oxygen species, which in turn affect membrane integrity by reacting membrane lipids. Oxidation of lipids (lipids peroxidation) in cell membranes significantly change the chemical and physiological properties of the membrane lipid bilayer, causing an increase in its permeability (Ozturk *et al*., 2012) [34]. Our results are coming in line with those of (Aazami *et al*.,2021) [35], since he found that increased salinity concentrations in the medium caused a significant damage in the cell membrane integrity indicators of tomato seedlings growing *in vitro*, including highly electrolyte leakage and increased content of malondialdehyde (MDA).

Conclusion

The results of this study revealed that salt stress evoked by the exposed to sodium chloride would negatively affects the growth of callus tissue in tomatoes. Additionally, high salt concentrations cause a significant reduction in vegetative growth, which in turn consider as evidenced by decreased fresh and dry weights.

Conversely, the tissue that showed a compensatory physiological response, characterized by a significant increased accumulation of proline and carbohydrates, reflecting their role in proofing the cell's capability for adaptation the osmotic stress. Furthermore, the high level of electron leakage may indicates a gradual deterioration the integrity of cell membrane in response to exposure to high salinity. Totally, these results claimed that salinity stresses callus cells and limit their growth, while their relative tolerance depends on physiological mechanisms that attempt to mitigate the effects of salt stress. Finally, further studies are highly recommended to investigate the molecular mechanisms participating in salt tolerance in tomatoes plant tissues.

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References

1. Abreu IA, Farinha AP, Negrão S, Gonçalves N, Fonseca C, Rodrigues M, *et al*. Coping with abiotic stress: proteome changes for crop improvement. J Proteomics. 2013;93:145-168.
2. Albaladejo I, Meco V, Plasencia F, Flores FB, Bolarin MC, Egea I. Unravelling the strategies used by the wild tomato species *Solanum pennellii* to confront salt stress: from leaf anatomical adaptations to molecular responses. Environ Exp Bot. 2017;135:1-12.
3. Benderradji L, Brini F, Kellou K, Ykhlef N, Djekoun A, Masmoudi K, *et al*. Callus induction, proliferation, and plantlets regeneration of two bread wheat (*Triticum aestivum* L.) genotypes under saline and heat stress conditions. Int Scholarly Res Notices. 2012;2012:367851.
4. Bhatia P, Ashwath N, Senaratna T, Midmore D. Tissue culture studies of tomato (*Lycopersicon esculentum*). Plant Cell Tissue Organ Cult. 2004;78(1):1-21.
5. Broadley MR, White PJ. Some elements are more equal than others: soil-to-plant transfer of radiocaesium and radiostrontium, revisited: Commentary on "Disparity in 90Sr and 137Cs uptake in Alpine plants: Phylogenetic effect and Ca and K availability". Plant Soil. 2012;355(1):23-27.
6. Chitlaru E, Pick U. Selection and characterization of *Dunaliella salina* mutants defective in haloadaptation. Plant Physiol. 1989;91(2):788-794.
7. Cuartero J, Bolarin MC, Asins MJ, Moreno V. Increasing salt tolerance in the tomato. J Exp Bot. 2006;57(5):1045-1058.
8. Flowers TJ, Munns R, Colmer TD. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. Ann Bot. 2015;115(3):419-431.
9. Geilfus CM. Chloride: from nutrient to toxicant. Plant Cell Physiol. 2018;59(5):877-886.
10. Hannachi S, Werbrouck S, Bahrini I, Abdelgadir A, Siddiqui HA, Van Labeke MC. Obtaining salt stress-tolerant eggplant somaclonal variants from *in vitro* selection. Plants. 2021;10(11):2539.
11. Hongqiao L, Suyama A, Mitani-Ueno N, Hell R, Maruyama-Nakashita A. A low level of NaCl stimulates plant growth by improving carbon and sulfur assimilation in *Arabidopsis thaliana*. Plants. 2021;10(10):2138.
12. Inculet CS, Mihalache G, Sellitto VM, Hlihor RM, Stoleru V. The effects of a microorganisms-based commercial product on the morphological, biochemical and yield of tomato plants under two different water regimes. Microorganisms. 2019;7(12):706.
13. Kacem NS, Delporte F, Muhovski Y, Djekoun A, Watillon B. *In vitro* screening of durum wheat against water-stress mediated through polyethylene glycol. J Genet Eng Biotechnol. 2017;15(1):239-247.
14. Khuder HH, Al-Taei YIH. Effect of salt stress on some growth indicators and cellular components of wheat (*Triticum aestivum* L.) callus. Int J Appl Agric Sci. 2015;1(4):91-94.
15. Klay I, Riahi L, Slim H. Variation in callus growth and *in vitro* regeneration among cultivated and wild wheat genotypes under increasing salt stress conditions. JAPS: J Anim Plant Sci. 2024;34(4).
16. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651-681.
17. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15(3).
18. Perez-Alfocea F, Guerrier G, Estan MT, Bolarin M. Comparative salt responses at cell and whole-plant levels of cultivated and wild tomato species and their hybrid. J Hortic Sci. 1994;69(4):639-644.
19. Rivera P, Moya C, O'Brien JA. Low salt treatment results in plant growth enhancement in tomato seedlings. Plants. 2022;11(6):807.
20. Sairam R, Tyagi A. Physiological and molecular biology of salinity stress tolerance in deficient and cultivated genotypes of chickpea. Plant Growth Regul. 2004;57(10):109-114.
21. Shrivastava P, Kumar R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci. 2014;22(2):123.
22. Taratima W, Chomarsa T, Maneerattanarungroj P. Salinity stress response of rice (*Oryza sativa* L. cv. Luem Pua) calli and seedlings. Scientifica. 2022;2022:5616683.
23. Tewary PK, Sharma A, Raghunath MK, Sarkar A. *In vitro* response of promising mulberry (*Morus* sp.) genotypes for tolerance to salt and osmotic stresses. Plant Growth Regul. 2000;30(1):17-21.
24. Tsunekawa K, Shijuku T, Hayashimoto M, Kojima Y, Onai K, Morishita M, *et al*. Identification and characterization of the Na+/H+ antiporter Nhas3 from the thylakoid membrane of *Synechocystis* sp. PCC 6803. J Biol Chem. 2009;284(24):16513-16521.
25. Wijerathna-Yapa A, Hiti-Bandaralage J. Tissue culture—A sustainable approach to explore plant stresses. Life. 2023;13(3):780.
26. Yazıcılar B, Bezirganoglu I. Characterization of the SOS1, SERK1, and WEE1 conferring a defense response to salt stress in alfalfa (*Medicago sativa* L.) callus. J Plant Growth Regul. 2023;42(11):7257-7265.
27. Zhang L, Song L, Shao H, Shao C, Li M, Liu M, *et al*. Spatio-temporal variation of rhizosphere soil microbial abundance and enzyme activities under different vegetation types in the coastal zone, Shandong, China. Plant Biosyst. 2014;148(3):403-409.
28. Bezirganoglu I. Response of five triticale genotypes to salt stress in *in vitro* culture. Turk J Agric For. 2017;41(5):372-380.
29. Sairam RK, Rao KV, Srivastava GC. Differential response of wheat genotypes to long-term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 2002;163(5):1037-1046.
30. Mohamed AN, Ismail MR. Changes in organic and inorganic solutes of *in vitro* tomato cultivars under NaCl stress. Aust J Crop Sci. 2011;5(8):939-944.
31. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids. 2008;35(4):753-759.
32. Szabados L, Savouré A. Proline: a multifunctional amino acid. Trends Plant Sci. 2010;15(2):89-97.

33. Zhou H, Shi H, Yang Y, Feng X, Chen X, Xiao F, *et al*. Insights into plant salt stress signaling and tolerance. J Genet Genomics. 2024;51(1):16-34.
34. Ozturk LD, Yavaz K, Ali UI, Ilhami D. Effects of long-term salt stress on antioxidant system, chlorophyll and proline in pea leaves. Rom Biotechnol Lett. 2012;17(3):7227-7236.
35. Aazami MA, Rasouli F, Ebrahimzadeh A. Oxidative damage, antioxidant mechanism and gene expression in tomato responding to salinity stress under *in vitro* conditions and application of iron and zinc oxide nanoparticles on callus induction and plant regeneration. BMC Plant Biol. 2021;21(1):597.