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Research Article

EFFECTS OF *IN VITRO* SALINITY STRESS ON GROWTH, OSMOLYTE ACCUMULATION, PHOTOSYNTHETIC ACTIVITY, AND MEMBRANE STABILITY OF BLACK CHERRY TOMATO (*Solanum lycopersicum* var. cerasiforme)

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ABSTRACT

Rising soil salinity poses a major challenge to global tomato production because it impairs germination, seedling development, and ultimately yield. Therefore, this study investigated the effects of NaCl (0–6 g/L) on germination, growth, and biochemical parameters of the black cherry tomato under in vitro conditions in the seedling stage. Germination percentage declined significantly with increasing salt concentration, from 100% at 0 g/L to 80% at 3 g/L, and seedling growth was strongly inhibited at 6 g/L. Moreover, seedling growth parameters, including fresh and dry biomass, root number and length, leaf number, leaf size, and plant height, were reduced as salinity increased. Biochemical analyses indicated that proline content increased concomitantly with declines in photosynthetic rate, total chlorophyll, relative water content, and membrane stability index ($p < 0.05$). Pearson correlation analysis confirmed a strong negative relationship between NaCl concentration and all measured traits ($r = –0.90$ to $–0.99$), and a strong positive correlation among germination, growth, and physiological variables ($r = 0.93–0.99$).

Keywords: black cherry tomato; membrane stability; osmolyte accumulation; photosynthetic performance; salinity stress

1. Introduction

Tomato (*Solanum lycopersicum* L.) is widely cultivated across the globe and remains a crop of considerable economic and nutritional importance (Kumar et al., 2020). Among its variants, black cherry tomato (*Solanum lycopersicum* var. *cerasiforme*), a small-fruited type within the Solanaceae, is commonly produced under both field and protected growing conditions. In Vietnam, this cultivar has recently gained attention as a premium export

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product, largely due to its distinctive appearance and flavor, which appeal strongly to international markets (Vuong et al., 2024). Beyond its market value, black cherry tomato also offers notable nutritional benefits. It supplies key vitamins, including A and C, and contains substantial amounts of bioactive carotenoids, particularly lycopene. This compound is known for its antioxidant capacity and has been associated with a reduced risk of certain cancers and cardiovascular diseases (Saini et al., 2020; Yang et al., 2023).

Despite these benefits, tomato cultivation increasingly faces constraints from abiotic stresses associated with climate change and environmental deterioration. In the Mekong Delta of Vietnam, salinity intrusion has intensified in recent years, posing a serious threat to agricultural systems. By 2023, over 200,000 hectares of farmland were affected by salinity levels unsuitable for optimal crop growth (Loc et al., 2021; Thach et al., 2023). Increased soil salinity elevates osmotic pressure in the rhizosphere, restricting water uptake by roots while transpiration persists. This imbalance induces a state commonly referred to as physiological drought, which negatively impacts plant growth and yield (Ali et al., 2025). In parallel, salt stress disrupts photosynthetic activity by inhibiting chlorophyll synthesis and promoting the accumulation of toxic ions. It also enhances respiratory consumption of carbohydrate reserves, thereby reducing energy availability at the cellular level and impairing normal physiological functions (Ali et al., 2025).

Several studies have investigated how tomatoes respond to salinity under in vitro conditions. Sané et al. (2021), for instance, demonstrated that NaCl exposure markedly affects vegetative growth across different tomato cultivars. Other work has focused on the composition of bioactive compounds in cherry tomatoes under diverse cultivation conditions, offering valuable insights into fruit quality (Rapa et al., 2021). However, these studies have not examined critical physiological adjustments, particularly osmotic regulation and photosynthetic performance, under controlled salt stress in vitro. While individual physiological and biochemical responses to salinity have been widely reported, studies that integrate multiple parameters remain limited. Notably, no research has simultaneously assessed growth characteristics, proline accumulation, chlorophyll content, photosynthetic activity, relative water content (RWC), and membrane stability in black cherry tomato under defined in vitro salinity conditions. This study, therefore, aims to address this gap by using in vitro culture systems to: (1) characterize growth and physiological responses of black cherry tomato seedlings exposed to different NaCl concentrations; (2) evaluate changes in proline levels and chlorophyll content; (3) assess photosynthetic performance and RWC; and (4) examine membrane stability.

2. Materials and methods

2.1. Plant material

Rang Dong Seed Company Limited (Ho Chi Minh City, Vietnam) provided black cherry tomato seeds (*Solanum lycopersicum* L. var. *cerasiforme*) for this study. Before culture initiation, we stored the seeds in sealed containers at 4 °C until use.

2.2. Culture medium and treatments

The study prepared Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar. The pH was adjusted to 6.0 before sterilization by autoclaving at 121 °C for 30 minutes. Sodium chloride (NaCl, 99.5%; Merck, Germany) was then incorporated into the medium at concentrations of 0 (control), 1, 2, 3, 4, 5, and 6 g/L to establish the salinity treatments.

2.3. Seed sterilization and inoculation

The seeds were initially agitated in a 1% (v/v) laboratory detergent solution for 5 minutes and then rinsed thoroughly with sterile distilled water. Surface sterilization was subsequently carried out following the protocol described by Raza et al. (2020). Briefly, seeds were immersed in 70 % (v/v) ethanol (Merck, Germany) for 1 min, then transferred to 5% (v/v) sodium hypochlorite solution (Thermo Fisher Scientific, USA) for 10 min, and finally rinsed 5 times with sterile distilled water. Sterile seeds were aseptically transferred (one seed per test tube) into 25 mL of MS medium containing the designated NaCl concentration. Each treatment comprised 10 test tubes and was replicated 3 times.

2.4. Culture conditions

All cultures were maintained in a growth chamber under a 12-h photoperiod (2,500 ± 500 lux), at 25 ± 2 °C, and 70 ± 5 % relative humidity. Cultures were observed under ambient laboratory lighting for germination assessments.

2.5. Growth traits and biochemical parameters analysis

2.5.1. Germination rate (%)

Germination rate as recorded weekly from weeks 1 to 4 (black tomato seeds were identified as germinated with an extended radicle for at least 1 mm), calculated as:

$$\text{Germination rate (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

2.5.2. Growth parameters

Growth parameters of *in vitro* black cherry tomato seedlings from weeks 1 to 8 were measured by methods described by Luong et al. (2023, 2025).

- Plant height (cm): measured from stem base to apex using a digital caliper (KY-21S, Kynup, China).
- Number of leaves: total count per seedling.
- Leaf length and width (cm): leaf length measured from the lamina base to tip; width measured at the widest midpoint by using a digital caliper.

- Leaf area (cm^2): scanned with a Canon LiDE 210 scanner and quantified using Fiji image-analysis software.
- Root number and length (cm): roots were washed free of agar, counted, and the longest root was measured with the digital caliper.
- Biomass: fresh weight determined immediately after removal from medium using an analytical balance (Sartorius TE214S), then dried at 60 °C for 24 h to constant weight to obtain dry weight.

2.5.3. Biochemical parameters

At week 8, five leaf samples were collected for biochemical analysis (the third leaf of each seedling was selected; if the third leaf was not available, all leaves were collected) as follows:

- Proline content (µmol/g FW) was extracted from the 3rd leaf, reacted with acid ninhydrin, and absorbance was measured at 520 nm. To prepare acid ninhydrin, 1.25 g of ninhydrin ($C_6H_4COCOCOH_2O$, BDH, AnalaR, England) was dissolved in 30 mL of glacial acetic acid, followed by the addition of 20 mL of 6 M orthophosphoric acid (H_3PO_4, Sigma-Aldrich, USA). Proline content was quantified using a standard curve (Paquin & Lechasseur, 1979)
- Photosynthetic intensity (µmol O_2 dm^{-2}/h) was measured using an LD2 oxygen electrode (Hansatech, UK). Leaf discs (≤10 cm^2) were exposed to white LED light (150 µmol m^{-2} s^{-1}, 2 000 lux) at 27 ± 0.2 °C. Oxygen exchange was recorded over 10 min; rates were calculated from minutes 4–7 (Luong et al., 2023).
- Chlorophyll content (mg/g FW) was extracted from 100 mg leaves in 5 mL of 80 % acetone for 2 h in the dark. After several rinses with 80% acetone, the residue was transferred to a test tube, and the volume was made up to 10.0 mL with 80% acetone. The extract was centrifuged at 1763 × g for 10 min (1580R, Labogene) and the supernatant absorbance was read at 663.2 nm (for Chl_a) and 646.8 nm (for Chl_b) (UV-1800, Shimadzu, Kyoto, Japan). The total chlorophyll content was calculated based on the equation described by Roshanak et al. (2016).
- Relative water content (RWC, %) was measured following Barrs and Weatherley (1962). Leaf fresh weight (FW) was recorded, then samples were soaked in distilled water for 4 h to obtain turgid weight (TW), dried at 60 °C for 24 h to obtain dry weight (DW). RWC was calculated as $\text{RWC}\ (\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$ (Sakya et al., 2018).
- Membrane stability index (MSI, %) was determined by incubating 100 mg leaf tissue in 10 mL distilled water at 40 °C for 30 min (initial conductivity, C1), then boiling at 100 °C for 10 min (final conductivity, C2). C1 and C2 values were determined with an electrical conductivity meter (Metrohm, Schweiz). MSI was calculated as $\text{MSI}\ (\%) = (1 - \frac{\text{C1}}{\text{C2}}) \times 100$ (Sakya et al., 2018).

2.6. Statistical analyses

We first assessed data normality using the Shapiro–Wilk test to verify the suitability of parametric analysis. It then applied one-way ANOVA to detect significant differences among NaCl treatments, followed by Duncan's multiple range test for post hoc comparison of means. Pearson correlation coefficients were calculated to examine relationships among growth performance, proline content, chlorophyll concentration, photosynthetic rate, RWC, and MSI. All statistical analyses were performed in IBM SPSS Statistics v. 26 (IBM Corp., Armonk, NY, USA), with significance defined at $p \leq 0.05$. Graphical data presentation and visualization were generated using OriginPro 2021b (OriginLab Corp., Northampton, MA, USA), and results are reported as mean ± standard deviation (SD).

3. Results and discussion

3.1. Germination rate

Germination declined progressively with rising NaCl levels (Table 1). Duncan's multiple range test further delineated seven statistically distinct subsets by week 4. The control (0 g/L) groups exhibited the highest germination (100 %) and differed ($p \leq 0.05$) from all salt treatments. Germination rate decreased dose-dependently; treatment NaCl 1 and 2 g/L attained 93 % and 87 %, respectively. At 3 g/L, germination peaked at 80 %, but higher salinities (4 g/L) yielded only 44 %, and 5 g/L just 18 %. No germination occurred at 6 g/L throughout the 4 weeks.

Table 1. *Germination rate (%) of black cherry tomato seeds under varying in vitro salt stress conditions over four weeks*

NaCl concentration (g//L)	Time (week)			
	1	2	3	4
0	88.89 ± 1.92^{a}	94.44 ± 1.93^{a}	97.78 ± 1.92^{a}	100.00 ± 0.00^{a}
1	83.33 ± 3.33^{b}	85.56 ± 1.93^{b}	90.00 ± 3.33^{b}	93.33 ± 3.34^{b}
2	75.55 ± 3.85^{c}	78.89 ± 1.92^{c}	85.56 ± 1.93^{c}	86.67 ± 3.33^{c}
3	51.11 ± 1.92^{d}	61.11 ± 1.92^{d}	72.22 ± 1.92^{d}	80.00 ± 3.33^{d}
4	0.00 ± 0.00^{e}	22.22 ± 1.92^{d}	32.22 ± 1.92^{e}	44.44 ± 1.93^{e}
5	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	17.78 ± 1.92^{f}	17.78 ± 1.92^{f}
6	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{g}	0.00 ± 0.00^{g}

Data were presented as mean ± standard deviation. Different letters in a column indicate significant differences between treatments with $p < 0.05$

3.2. Growth parameters

In general, increasing salinity led to a progressive and significant inhibition of all measured growth parameters (dry weight, fresh weight, root length, number of roots, leaf area, leaf width, leaf length, number of leaves, and plant height) across the four sampling dates (week 2, 4, 6, and 8) (Figure 1). At week 2, seedlings grown in the absence of NaCl (0 g/L) exhibited the greatest biomass accumulation, whereas even mild salinity (2 g/L) caused a marked decline ($p < 0.05$). Moreover, root elongation and root branching were already

compromised at 1 g/L ($p < 0.05$), and the seedlings failed to produce measurable biomass or root systems at ≥ 4 g/L.

By week 4, this inhibitory effect persisted, although seedlings at 1 g/L still maintained approximately 97 % of the control dry weight (not significantly different), higher salinities (≥ 2 g/L) triggered significant reductions in both shoot and root parameters ($p < 0.05$). Furthermore, leaf area and plant height were already halved at 2 g/L compared with the control. In contrast, treatments ≥ 4 g/L again led to a complete cessation of detectable growth.

Interestingly, at weeks 6 and 8, the deleterious impact of even low-level salinity became more pronounced over time. For instance, by week 6, dry weight under 1 g/L NaCl was significantly reduced ($p < 0.05$), and root length decreased by more than 15 %. Moreover, the number of leaves and leaf area declined markedly at 2 g/L NaCl ($p < 0.05$). By week 8, these trends culminated in severely stunted plants: fresh weight at 1 g/L NaCl dropped to 201.60 g (−10 % relative to control), whereas even moderate salinity (2–4 g/L) reduced plant height by over 50 % and leaf area by more than 30 % ($p < 0.05$).

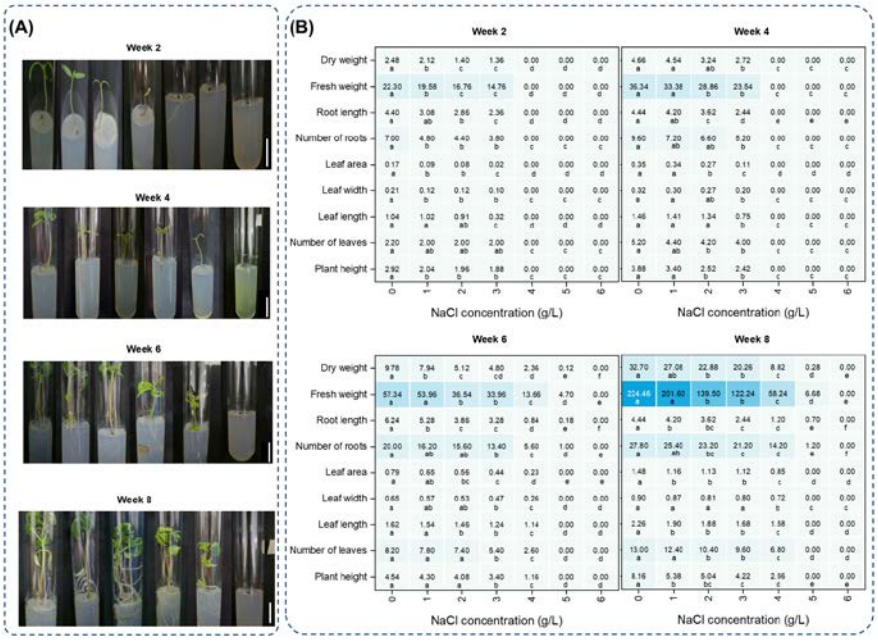

Figure 1. *Effect of NaCl concentration on seedling morphology and growth. (A) Representative seedlings in test tubes at 2, 4, 6, and 8 weeks post-treatment (left to right: 0 → 5 g/L); scale bar = 1 cm. (B) Growth-related parameters measured at weeks 2, 4, 6, and 8. Different letters denote significant differences among treatments ($p \leq 0.05$).*

3.3. Biochemical parameters

NaCl had a concentration-dependent effect on biochemical parameters of black cherry tomato under *in vitro* conditions (Figure 2). Proline accumulation increased progressively with salinity up to 4 g/L. Under control conditions (0 g/L), proline was 13.11 ± 0.98 µmol/g FW; it rose to 19.57 ± 2.14 µmol/g FW at 1 g/L and peaked at 85.43 ± 2.91 µmol/g FW by 4 g/L, each increment being statistically distinct ($p \leq 0.05$). Contrary to photosynthetic

intensity, which declined in a nearly mirror-image pattern, dropping from 5.70 ± 0.27 μmol O_2 dm^{-2}/h in the control to 2.40 ± 0.73 μmol O_2 dm^{-2}/h at 4 g/L (all pairwise differences $p \leq 0.05$). Likewise, total chlorophyll content fell significantly from 3.57 ± 0.08 mg/g FW (0 g/L) to 1.22 ± 0.08 mg/g FW at the highest tested salinity.

Moreover, salinity stress impaired water balance and membrane integrity. RWC decreased from 94.95 ± 1.67 % at 0 g/L to 59.83 ± 2.37 % at 4 g/L NaCl, while the MSI dropped from 87.25 ± 1.52 % to 51.76 ± 2.08 % over the same range, with all differences being significant ($p \leq 0.05$). At 5 and 6 g/L NaCl, no viable tissue remains for measurement.

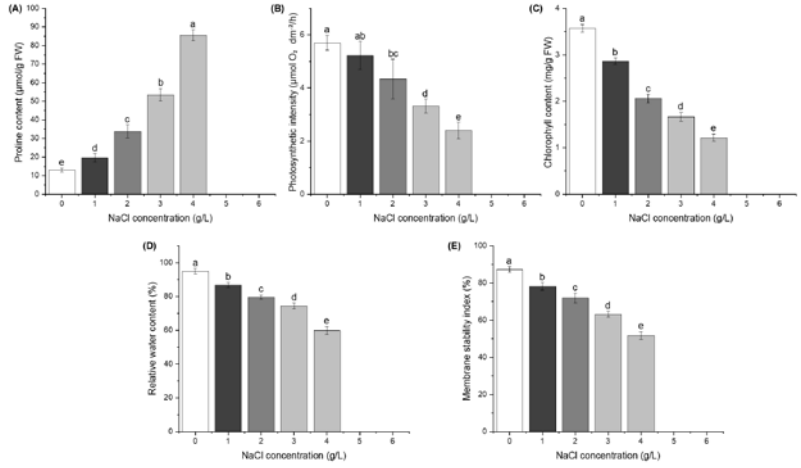

Figure 2. *Effects of NaCl concentration on biochemical parameters of in vitro–cultured black cherry tomato (A) proline content, (B) photosynthetic intensity, (C) chlorophyll content, (D) relative water content (RWC), and (E) membrane stability index (MSI). Different letters denote significant differences at $p \leq 0.05$*

3.4. Pearson correlation

Pearson correlation analysis (Figure 3) revealed that NaCl concentration was uniformly and highly negatively associated with germination rate ($r = –0.95$, $p < 0.05$), plant height ($r = –0.96$), number of leaves ($r = –0.98$), leaf length ($r = –0.98$), width ($r = –0.97$), and leaf area ($r = –0.99$). Similar strong negative correlations were observed with root number ($r = –0.96$), root length ($r = –0.97$), fresh weight ($r = –0.99$), dry weight ($r = –0.95$), RWC ($r = –0.94$), photosynthetic intensity ($r = –0.90$), MSI ($r = –0.95$), and chlorophyll content ($r = –0.94$). In contrast, germination rate was very strongly and positively correlated with all growth and physiological metrics ($r = 0.93–0.99$, $p < 0.05$), indicating that reductions in germination under higher salinity were tightly linked to declines in seedling vigor, osmotic adjustment, photosynthetic performance, and membrane integrity.

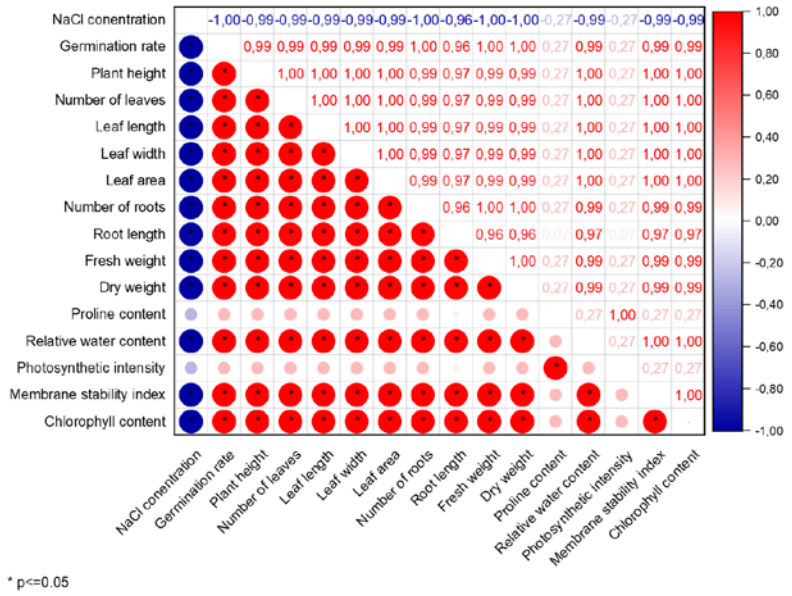

Figure 3. *Pearson correlation matrix of NaCl concentration versus germination rate, growth parameters, and physiological–biochemical traits of in vitro–cultured black cherry tomato. Asterisks (*) denote correlations significant at $p \leq 0.05$*

3.4. Discussion

The results reveal a clear, dose-dependent decline in seed germination of black cherry tomato as NaCl concentration increased under in vitro conditions. This reduction arises from the combined influence of osmotic stress and ionic toxicity. Higher salinity lowers the water potential of the medium, thereby limiting seed imbibition, while excess Na^+ and Cl^- ions compromise membrane stability and disrupt enzymatic activities required for radicle emergence (Arif et al., 2020). In tomato, germination typically decreases even at relatively low salinity levels (approximately 50–100 mM NaCl) and becomes markedly restricted at concentrations above 150–200 mM (López-Méndez et al., 2024; Roșca et al., 2023). Under such conditions, failure to germinate often reflects complete inhibition of radicle protrusion rather than a simple delay in development.

Restricted water uptake under saline conditions impairs the generation of turgor pressure necessary for embryo expansion, while ionic toxicity interferes with the mobilization of stored carbohydrates and the activity of key hydrolytic enzymes, including α-amylase (Liu et al., 2018). In addition, recent findings suggest that NaCl exposure may suppress the expression of genes associated with amylase synthesis and reduce levels of bioactive gibberellins, further limiting starch breakdown and embryo growth (Liu et al., 2022; Xiong et al., 2024). Successful germination depends on coordinated endosperm weakening and sufficient embryo turgor (Chandrasekaran et al., 2022); disruption of either process can effectively prevent radicle emergence (Wahid et al., 1999; Ali & Elozeiri, 2017). The progressive reduction in germination observed in this stuy, therefore, reflects the combined inhibitory effects of salinity on both water uptake and metabolic activation.

In addition to its effects on germination, salinity markedly reduced all measured growth parameters, even at relatively low concentrations (1–2 g/L), while growth was completely inhibited at ≥ 4 g/L. By the fourth week, seedlings exposed to 2 g/L NaCl showed reductions of approximately 50% in both leaf area and plant height. These declines became more pronounced by week 8 under moderate salinity levels (2–3 g/L). Such patterns indicate that salt stress rapidly disrupts key processes governing cell division and expansion (Alharbi et al., 2022; Shahid et al., 2020).

Early decreases in root length and branching at 1–2 g/L NaCl further suggest impaired meristem function and disturbances in auxin transport, consistent with previous in vitro studies on tomato (Sootahar et al., 2024). At the cellular level, the accumulation of Na^+ and Cl^- likely interferes with cell expansion, alters hormonal regulation, particularly auxin and cytokinin signaling, and suppresses mitotic activity in meristematic tissues (Arif et al., 2020; Hasanuzzaman et al., 2012). These effects become progressively more severe over time as ionic toxicity increases and nutrient uptake is further restricted, ultimately limiting overall plant growth and vigor.

At the biochemical level, proline content increased progressively with rising salinity, whereas photosynthetic efficiency and total chlorophyll content declined in parallel (Singh et al., 2015). This opposing trend highlights the role of proline as both an osmoprotectant and a stress-induced metabolite. Comparable responses have been reported by Sané et al. (2021), who observed increased proline accumulation accompanied by chlorophyll degradation in tomato seedlings exposed to PEG-induced water stress, linking proline accumulation to osmotic adjustment and reactive oxygen species (ROS) scavenging. Similarly, Kahlaoui et al. (2014) showed that salt-tolerant tomato cultivars maintained higher chlorophyll fluorescence and exhibited slower pigment loss under saline conditions, indicating greater stability of thylakoid membranes and more efficient antioxidant systems. Salinity stress also had pronounced effects on plant water status and membrane integrity, as reflected by decreases in RWC and MSI. The higher osmotic pressure of the saline medium reduces its water potential, thereby restricting water uptake by roots and lowering tissue hydration (Lutts et al., 2004). At the same time, ionic disturbances, particularly the accumulation of Na^+ alongside the displacement of K^+ and Ca^{2+}, promote oxidative stress, which in turn leads to lipid peroxidation of cellular membranes (Chakraborty et al., 2018). This damage manifests as increased membrane permeability and reduced MSI values.

Similar patterns have been documented by Bogoutdinova et al. (2024), who reported that salt-sensitive tomato genotypes experienced reductions of 30–40% in RWC and over 40% loss in MSI under 100 mM NaCl *in vitro*. Correlation analysis in the present study further reinforces these findings, showing strong negative relationships between NaCl concentration and germination ($r = –0.95$), growth parameters ($r = –0.96$ to $–0.99$), and physiological traits ($r = –0.90$ to $–0.97$). In contrast, germination was positively correlated with seedling vigor, proline accumulation, photosynthetic performance, and membrane stability ($r = 0.93$–0.99), underscoring the integrated nature of plant responses to salinity stress.

4. Conclusion

Increasing NaCl concentrations exerted a strong inhibitory effect on germination, growth, and physiological performance in black cherry tomato. Germination declined markedly at concentrations above 3 g/L and was completely suppressed at 6 g/L. Seedling development, including biomass accumulation, and root and leaf formation, was already adversely affected at 1–2 g/L and became entirely inhibited at ≥ 4 g/L. Concurrently, proline content increased, whereas photosynthetic rate, chlorophyll content, RWC, and MSI showed consistent declines under saline conditions.

Correlation analysis confirmed strong negative associations between NaCl concentration and all measured variables, along with strong positive relationships among germination, growth, and physiological parameters. These results highlight the tightly interconnected nature of plant responses to salinity stress. Future work should prioritize the identification of salt-tolerant genotypes through combined physiological and molecular approaches, as well as the evaluation of exogenous osmoprotectants or biostimulants to enhance tomato performance under saline conditions in both controlled environments and field settings.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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ẢNH HƯỞNG CỦA STRESS MẶN *IN VITRO* ĐẾN SỰ TĂNG TRƯỞNG, TÍCH LŨY THẨM THẤU, HIỆU SUẤT QUANG HỢP VÀ ĐỘ ỔN ĐỊNH MÀNG Ở CÀ CHUA BI ĐEN (*Solanum lycopersicum* var. cerasiforme)

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TÓM TẮT

Độ mặn đất tăng cao đặt ra thách thức to lớn đối với quá trình sản xuất cà chua toàn cầu, do độ mặn làm suy yếu quá trình nảy mầm, sự phát triển của cây con và sản lượng. Nghiên cứu này được thực hiện nhằm khảo sát tác động của NaCl (0–6 g/L) lên các thông số nảy mầm, sinh trưởng, và sinh hóa của cà chua bi đen trong điều kiện in vitro. Tỉ lệ nảy mầm giảm đáng kể theo nồng độ muối, từ 100% ở nồng độ 0 g/L xuống 80% ở nồng độ 3 g/L và ức chế sinh trưởng sau nảy mầm ở nồng độ 6 g/L. Các thông số sinh trưởng của cây con, bao gồm sinh khối tươi và khô, số lượng và chiều dài rễ, số lượng lá, kích thước và chiều cao cây giảm. Phân tích sinh hóa cho thấy hàm lượng proline tăng với sự suy giảm về tốc độ quang hợp, tổng diệp lục, hàm lượng nước tương đối và chỉ số ổn định màng ($p < 0,05$). Phân tích tương quan Pearson xác nhận mối liên hệ tiêu cực mạnh giữa nồng độ NaCl và tất cả các đặc điểm được đo ($r = –0,90$ đến $–0,99$) và mối tương quan tích cực mạnh giữa các số liệu nảy mầm, tăng trưởng và sinh lí ($r = 0,93–0,99$).

Từ khóa: cà chua bi đen; ổn định màng tế bào; tích lũy chất điều hòa thẩm thấu; hiệu suất quang hợp; căng thẳng mặn