

Original article

Proline-Mediated Growth Regulation and Antioxidant Defense Enhancement in Lettuce under Salinity Stress

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ABSTRACT

Keywords:

Proline, Salinity Stress, Lettuce, Antioxidant Enzymes, Photosynthetic Pigments, Ionic Balance.

This study investigated the mitigating effects of exogenous proline application on lettuce (*Lactuca sativa* L.) plants subjected to salinity stress induced by NaCl. The experiment evaluated vegetative growth, photosynthetic pigments, chemical constituents, and antioxidant enzyme activity under varying proline concentrations (with 200 mg/l identified as the optimal treatment) and salinity conditions. Salt stress significantly impaired vegetative growth, reducing fresh weight by 6.73%, leaf number by 10.0%, and leaf area by 19.08% compared to control conditions; however, dry matter content increased under salinity. Foliar application of proline effectively alleviated these adverse effects, with 200 mg/l proline achieving the highest plant yield under non-saline conditions (5.00% increase over control) and substantially offsetting growth inhibition under NaCl stress. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were significantly degraded by salinity, yet proline-treated plants exhibited marked improvement. The 200 mg/l proline treatment enhanced total chlorophyll and carotenoid contents by approximately 9.34% and 9.77%, respectively, relative to untreated controls. Regarding chemical constituents, NaCl stress reduced nitrogen (N), phosphorus (P), potassium (K), and the K/Na ratio while increasing sodium (Na⁺) and chloride (Cl⁻) accumulation. Proline application, particularly at 200 mg/l, counteracted these ionic imbalances by elevating N, P, K, and K/Na levels and suppressing Na⁺ and Cl⁻ uptake. Antioxidant enzyme activities—peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT)—were significantly stimulated under salinity, increasing by 45.87%, 113.1%, and 58.5%, respectively, compared to control plants. Proline spraying further enhanced these enzymatic activities, reinforcing the plant's oxidative stress defense mechanism. In conclusion, foliar application of proline at 200 mg/l represents an effective strategy to ameliorate salinity-induced damage in lettuce by improving growth parameters, photosynthetic efficiency, ionic homeostasis, and antioxidant capacity.

Introduction

Lettuce (*Lactuca sativa* L.) constitutes one of the most economically important leafy vegetables worldwide, primarily consumed in fresh or minimally processed forms such as salads [1]. This crop is nutritionally distinguished by its substantial content of vitamins, minerals, dietary fiber, and omega-3 fatty acids, all of which are indispensable for normal human growth and metabolic functions. Additionally, lettuce serves as a rich source of bioactive phytochemicals, including phenolic compounds, tocopherols, ascorbic acid, and lignans, thereby exhibiting notable therapeutic properties such as anti-inflammatory, hypocholesterolemic, and antidiabetic activities [2].

Despite its nutritional significance, lettuce is classified among vegetable species with moderate to high sensitivity to salinity stress [3], a constraint that severely compromises its agronomic performance and marketable yield. Salinity represents a pervasive abiotic stressor that substantially undermines agricultural productivity, particularly in arid and semi-arid agro-ecosystems where irrigation practices often exacerbate soil sodicity [4]. The continuous application of saline irrigation water has progressively converted fertile arable lands into non-productive areas, posing a critical threat to global food security [5]. At the physiological level, salinity stress manifests through dual mechanisms: osmotic stress, which reduces water availability to plant tissues, and ionic stress, arising from excessive accumulation of Na⁺ and Cl⁻ ions in the soil solution [6]. These perturbations collectively restrict root system development, impair mineral nutrient acquisition, induce lipid peroxidation and protein denaturation, destabilize membrane integrity, and suppress enzymatic activities essential for metabolic homeostasis [7]. Consequently, photosynthetic apparatus functionality

deteriorates, evidenced by chlorophyll degradation, diminished leaf area, and the onset of necrotic lesions and premature leaf abscission [8]. Furthermore, salinity stress interferes with growth regulatory processes, disrupts normal developmental patterns, and significantly alters the uptake and partitioning of essential mineral nutrients [9-12], while concurrently stimulating excessive generation of reactive oxygen species (ROS) [13].

To counteract salinity-induced cellular damage, plants activate an integrated network of protective mechanisms. These adaptive responses encompass: (i) upregulation of antioxidant enzymatic and non-enzymatic systems to attenuate ROS accumulation; (ii) biosynthesis and accumulation of compatible osmolytes to sustain tissue water potential and cellular turgor; and (iii) enhanced expression of regulatory genes governing ion transport, compartmentalization, and salt exclusion pathways [14,15]. Among these osmolytes, proline (an imino acid) has garnered considerable attention due to its multifunctional roles in stress physiology. Beyond its conventional function as an osmoprotectant, proline contributes to protein stabilization through hydrogen bonding, maintains membrane structural integrity, enhances cellular water retention capacity, and modulates enzyme activation under adverse conditions [16,17]. Moreover, proline exhibits potent ROS-scavenging capacity and reinforces antioxidative defense machinery, thereby protecting lipids, proteins, and nucleic acids from oxidative damage [18,19].

Although the protective efficacy of exogenous proline application has been documented across diverse crop species [20], its dose-dependent physiological and biochemical impacts on lettuce under controlled salinity regimes remain insufficiently characterized. Specifically, the optimal proline concentration required to maximize growth performance, preserve photosynthetic pigment composition, restore mineral ion homeostasis, and enhance antioxidant enzyme activity in salt-stressed lettuce has not been systematically quantified. Therefore, the present investigation was undertaken to evaluate the ameliorative potential of foliar-applied proline at varying concentrations on lettuce plants exposed to NaCl-induced salinity stress, with particular emphasis on vegetative growth attributes, photosynthetic pigment profiles, chemical constituents, and antioxidant enzyme responses.

Methods

Experimental Site and Design

A pot experiment was conducted at the experimental farm of the Faculty of Agriculture, Mansoura University, Egypt, to evaluate the effects of exogenous proline application on lettuce (*Lactuca sativa* L. cv. Nader) grown under NaCl-induced salinity stress. The study comprised six treatment combinations arranged in a randomized complete block design (RCBD) with three replicates. The factorial treatments included two salinity levels (0 and 50 mM NaCl) and three foliar proline concentrations (0, 100, and 200 mg/L).

Plant Material and Cultural Practices

Plastic pots (40 cm diameter) were filled with 10 kg of clay loam soil. A compound fertilizer (N:P:K 20:20:20) was applied four times at two-week intervals following transplanting. Seeds were surface-sterilized by immersion in 0.5% sodium hypochlorite solution for 4 min, subsequently rinsed thoroughly with distilled water, and sown in a nursery bed in mid-October 2023. Uniform seedlings were transplanted into the prepared pots during the first week of November 2023. Irrigation was administered three times at 10-day intervals, alternating between saline solution (twice) and tap water (once) to maintain the designated salinity regime. Foliar proline treatments (0, 100, and 200 mg/L) were applied three times at 14-day intervals commencing after transplanting.

Growth and Yield Measurements

Seventy days after transplanting, three plants per treatment were randomly selected and harvested for morphological and physiological analyses. The following parameters were recorded:

- Number of leaves per plant: counted manually.
- Plant fresh weight (g): determined using a digital balance.
- Dry matter percentage: calculated after oven-drying samples at 70 °C to constant weight.
- Leaf area (cm² plant⁻¹): estimated according to the method described by [21].

Photosynthetic Pigment Analysis

Chlorophyll a, chlorophyll b, and total chlorophyll contents (mg g⁻¹ fresh weight) were quantified following the spectrophotometric procedure of [22]. Fresh leaf tissue was homogenized in 80% acetone, filtered through Whatman No. 1 filter paper, and absorbance was measured using a UV-visible spectrophotometer. Carotenoid concentrations (mg g⁻¹ fresh weight) were determined spectrophotometrically according to [23].

Chemical Constituent Analysis

Dried leaf samples were analyzed for nitrogen (N), phosphorus (P), potassium (K), sodium (Na⁺), and chloride (Cl⁻) percentages using the standard analytical protocols outlined by [24].

Antioxidant Enzyme Assays

Leaf tissue (0.2 g) was homogenized in 4 mL of ice-cold 0.1 M sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% (w:v) polyvinylpyrrolidone. The homogenate was centrifuged at 10,000 × g for 20 min at 4 °C, and the resulting supernatant was utilized for enzyme activity determinations. Protein concentration in the extracts was estimated following [25]. Peroxidase (POD; EC 1.11.1.7) activity was assayed as described by [26] and expressed as units mg⁻¹ protein. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured by monitoring the inhibition of nitroblue tetrazolium (NBT) reduction at 560 nm according to [27]. Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the decline in H₂O₂ absorbance at 240 nm following the method of [28].

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) using SPSS statistical software (SPSS for Windows, SPSS Inc., Chicago, IL, USA). Treatment means were compared using Duncan's multiple range test at a significance level of P ≤ 0.05.

Results

Vegetative Growth and Yield

The results presented in (Figure 1) demonstrated that salinity stress significantly affected the vegetative growth and yield of lettuce plants. Compared with the non-saline control, exposure to NaCl reduced fresh weight by ****6.73%****, leaf number by ****10.00%****, and leaf area by ****19.08%****, whereas dry matter showed a significant increase under saline conditions. These findings indicate that salinity markedly suppressed plant growth, with leaf area being the most affected vegetative trait. Foliar application of proline improved the growth performance of lettuce plants under both normal and saline conditions. The greatest enhancement in plant yield was recorded with ****200 mg/L proline under 0 mM NaCl****, where fresh weight increased by ****5.00%**** compared with the untreated control grown under the same non-saline conditions. In addition, proline-treated plants exhibited greater leaf production and larger leaf area than the corresponding untreated plants exposed to salinity, indicating a substantial recovery of vegetative growth. Overall, the data confirm that salinity caused significant reductions in lettuce vegetative growth, particularly in leaf area (****19.08%****), followed by leaf number (****10.00%****) and fresh weight (****6.73%****). Conversely, foliar application of proline effectively mitigated these reductions, with the ****200 mg/L**** treatment producing the highest fresh biomass and improving overall plant performance, highlighting its effectiveness in enhancing lettuce productivity under saline conditions.

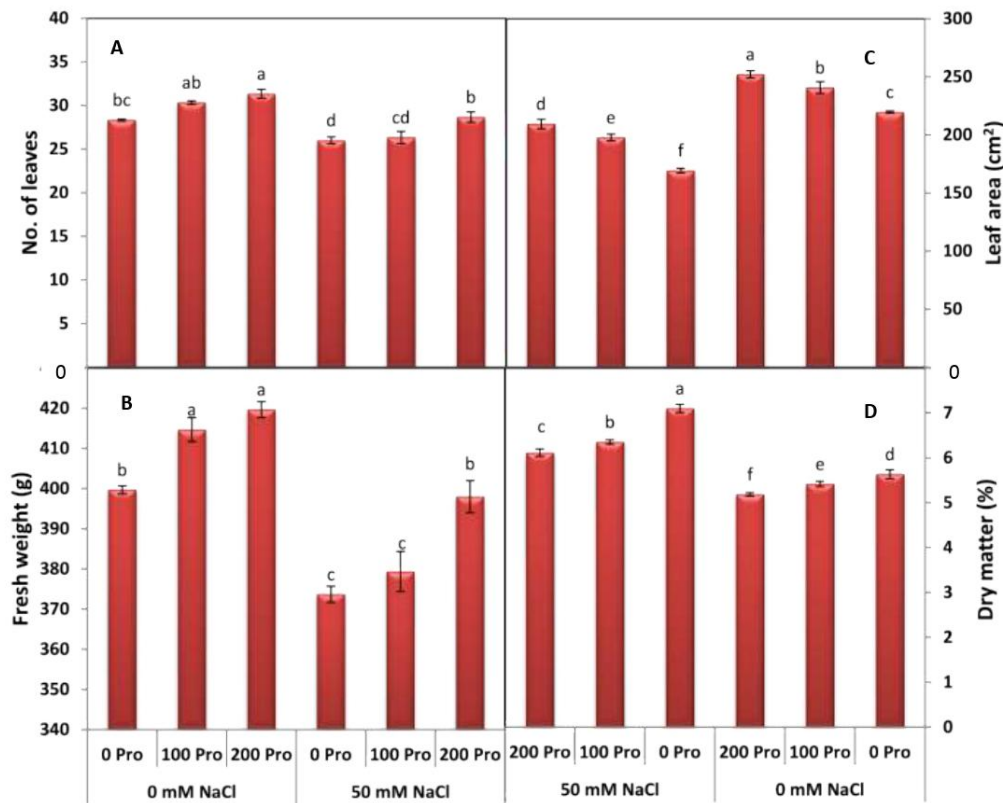


Figure 1. Impact of proline applications on the number of leaves plant⁻¹ (A), fresh weight (B), leaf area (C), and dry matter (D) of lettuce plants under salinity stress. Significant variation in relation to the Duncan test is indicated by diverse letters

Photosynthetic pigments

The results presented in (Figure 2) showed that salinity stress significantly reduced the concentrations of photosynthetic pigments in lettuce plants. Exposure to NaCl markedly decreased chlorophyll ****a****, chlorophyll ****b****, total chlorophyll, and carotenoid contents compared with plants grown under non-saline conditions, indicating that salinity negatively affected the photosynthetic apparatus. Foliar application of proline significantly improved the photosynthetic pigment content under both normal and saline conditions. Among the tested concentrations, ****200 mg /L proline**** produced the greatest enhancement, increasing ****total chlorophyll by 9.34%**** and ****carotenoids by 9.77%**** compared with the untreated control. In addition, chlorophyll ****a**** and chlorophyll ****b**** also showed higher values in proline-treated plants than in untreated plants exposed to salinity, demonstrating the beneficial effect of proline on maintaining pigment accumulation. Overall, the findings indicate that salinity markedly impaired photosynthetic pigment synthesis, whereas exogenous proline effectively alleviated these adverse effects. The ****200 mg/ L**** proline treatment was the most effective, resulting in the highest accumulation of photosynthetic pigments and confirming its positive role in preserving the photosynthetic capacity of lettuce plants under saline conditions.

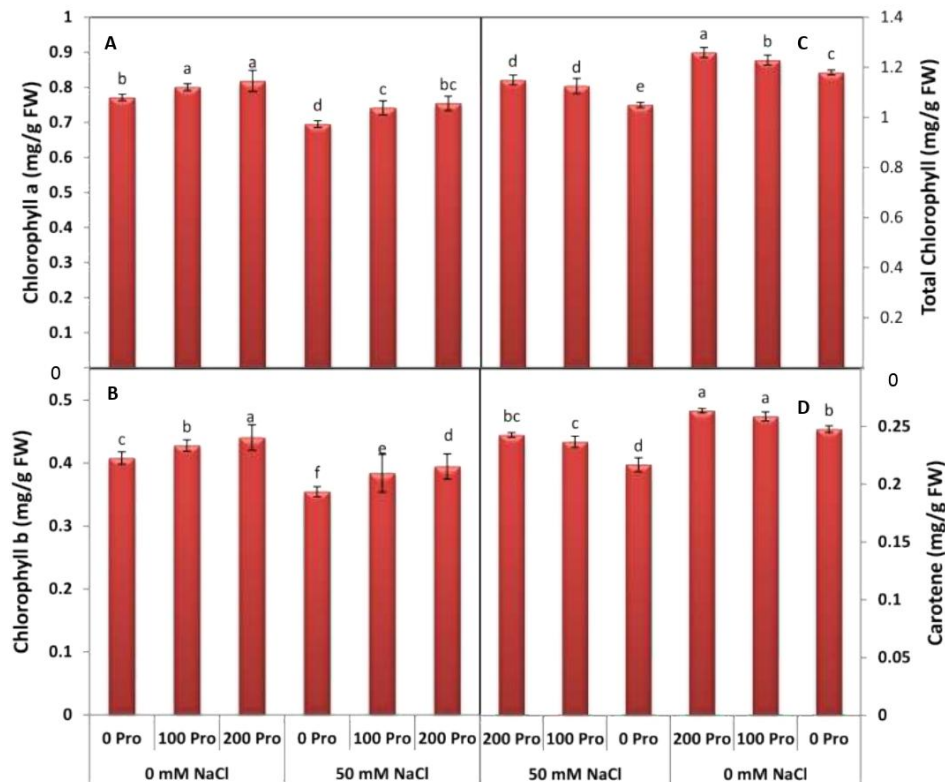


Figure 2. Impact of proline applications on chlorophyll a (A), total chlorophyll (B), chlorophyll b (C), and carotenoids (D) of lettuce plants under salinity stress. Significant variation in relation to the Duncan test is indicated by diverse letters

Chemical constituents

The results presented in (Figure 3) demonstrated that salinity stress significantly altered the mineral composition of lettuce plants. Exposure to NaCl resulted in marked reductions in the concentrations of **nitrogen (N)**, **phosphorus (P)**, **potassium (K)**, and the **K/Na ratio**, while significantly increasing the accumulation of **Na⁺** and **Cl⁻** compared with plants grown under non-saline conditions. These changes indicate a pronounced disturbance in nutrient balance under saline conditions. Foliar application of proline markedly improved the chemical composition of lettuce plants under salt stress. Proline-treated plants exhibited higher concentrations of **N**, **P**, **K**, and **K/Na ratio**, together with lower **Na⁺** and **Cl⁻** contents than untreated salt-stressed plants, indicating a substantial improvement in mineral status. Among the tested treatments, **200 mg /L proline** produced the most pronounced response, showing the highest values of beneficial mineral nutrients and the lowest accumulation of toxic ions. Overall, the results confirm that salinity significantly disrupted nutrient homeostasis by decreasing essential mineral elements and increasing the accumulation of **Na⁺** and **Cl⁻**. Exogenous application of proline effectively alleviated these adverse effects, with the **200 mg /L** treatment providing the greatest improvement in mineral balance and maintaining the highest **K/Na ratio**, thereby demonstrating its superior effectiveness in enhancing ionic regulation under saline conditions.

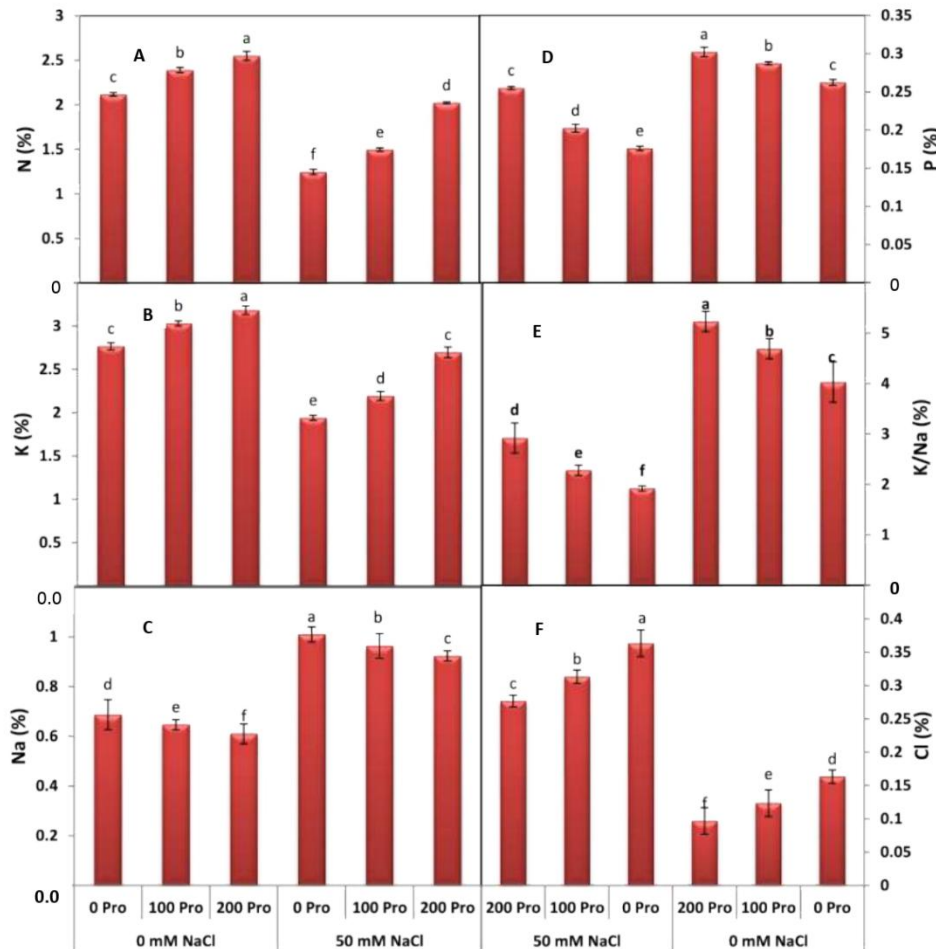


Figure 3. Impact of proline applications on N (A), K (B), Na⁺ (C), P (D), K/Na (E), and Cl⁻ (F) of lettuce plants under salinity stress. Significant variation in relation to the Duncan test is indicated by diverse letters.

Antioxidant enzymes

The results presented in (Figure 4) revealed that salinity stress significantly enhanced the activities of the antioxidant enzymes peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) in lettuce plants. Compared with the non-saline control, NaCl treatment increased POD activity by 45.87%, SOD activity by 113.10%, and CAT activity by 58.50%, with SOD exhibiting the greatest relative increase among the evaluated antioxidant enzymes. Foliar application of proline further promoted antioxidant enzyme activities under both normal and saline conditions. Proline-treated plants exhibited higher POD, SOD, and CAT activities than the untreated plants, indicating a stronger antioxidant response. The enhancement was more pronounced under salinity stress, demonstrating that proline application effectively stimulated the antioxidant defense system. Among the tested treatments, the 200 mg/L proline application produced the highest antioxidant enzyme activities, indicating its superior effectiveness in improving the enzymatic antioxidant capacity of lettuce plants. Overall, the findings demonstrate that salinity markedly increased antioxidant enzyme activities, with SOD (113.10%) showing the greatest response, followed by CAT (58.50%) and POD (45.87%). Exogenous application of proline further enhanced the activities of these enzymes, particularly at 200 mg/L, confirming its effectiveness in strengthening the antioxidant defense system and improving the physiological performance of lettuce plants under saline conditions.

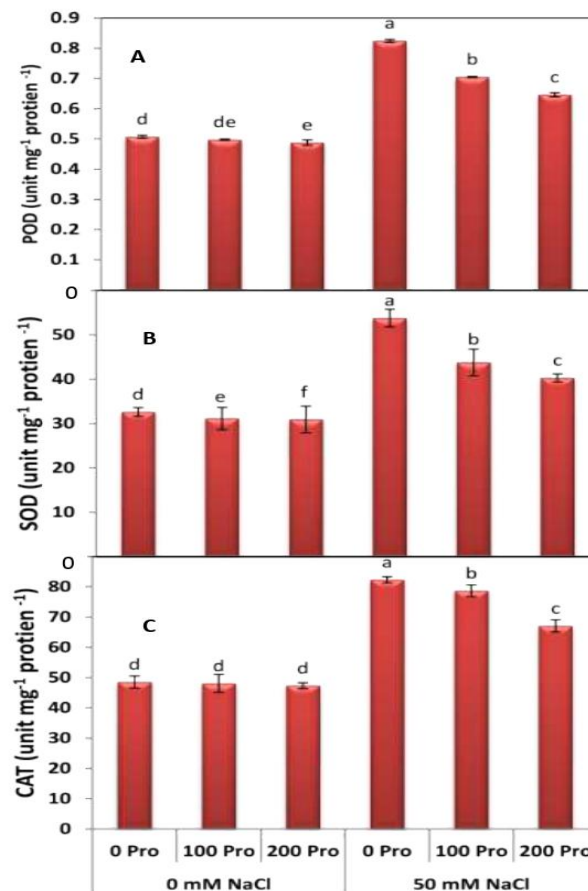


Figure 4. Impact of proline applications on POD (A), SOD (B), and CAT (C) of lettuce plants under salinity stress. Significant variation in relation to the Duncan test is indicated by diverse letters

Discussion

Salinity stress imposes severe constraints on plant growth and development by disrupting multiple physiological and biochemical processes. The present investigation revealed that lettuce plants exposed to 50 mM NaCl exhibited significant reductions in vegetative growth parameters, including leaf number, leaf area, and fresh biomass, accompanied by a concomitant increase in dry matter percentage. These findings align with previous reports indicating that salinity adversely affects plant growth, photosynthetic efficiency, water status, oxidative biomarkers, osmolyte accumulation, and antioxidant enzyme activity [29,30]. The growth inhibition observed under saline conditions may be attributed to the detrimental impacts of excess Na⁺ and Cl⁻ ions on fundamental physiological functions, including photosynthetic electron transport, stomatal conductance, reactive oxygen species (ROS) accumulation, and perturbations in nutritional and hormonal homeostasis [31-32]. The suppression of lettuce growth under salt stress can be further explained by impaired assimilate translocation through vascular conductive tissues, resulting in restricted cell division and elongation [33]. Additionally, salinity-induced enzymatic inhibition of chlorophyll biosynthesis pathways leads to diminished chlorophyll content [34], which subsequently compromises photosynthetic capacity and overall plant productivity.

Similar reductions in chlorophyll content and growth performance under saline conditions have been documented across diverse plant species [35-38]. Under non-stress conditions, plants maintain a delicate equilibrium between ROS generation and antioxidant defense capacity. However, salinity stress disrupts this balance, leading to excessive ROS accumulation that inflicts oxidative damage on cellular macromolecules. To mitigate this damage, plants have evolved sophisticated enzymatic and non-enzymatic antioxidant systems that strictly regulate intracellular ROS levels [39]. The enzymatic cascade initiated by superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals (O₂⁻) into hydrogen peroxide (H₂O₂) and molecular oxygen (O₂).

Subsequently, peroxidase (POD) and catalase (CAT) facilitate the detoxification of H₂O₂ into water and oxygen, thereby preventing the accumulation of this cytotoxic molecule [40]. The present results demonstrate that NaCl stress significantly upregulated the activities of POD, SOD, and CAT, indicating a

coordinated antioxidant response aimed at scavenging excess H₂O₂ and alleviating oxidative damage. This enzymatic cooperation is essential for effective ROS detoxification under salinity stress [31,41]. While certain antioxidant enzymes primarily function in growth regulation and detoxification processes, others are critical for maintaining ROS homeostasis and redox signaling [42,43].

Exogenous application of proline has emerged as an effective strategy for enhancing plant tolerance to various abiotic stressors through the modulation of both enzymatic and non-enzymatic antioxidant defense networks [44]. The current findings demonstrate that foliar proline supplementation, particularly at 200 mg/L, significantly ameliorated the adverse effects of salinity on lettuce growth and physiological performance. Proline-treated plants exhibited enhanced vegetative growth compared to both salt-stressed and unstressed control plants (Figure 1). These beneficial effects may be attributed to the elevated endogenous proline levels in plant tissues, which confer osmotic adjustment and cellular protection under saline conditions. Furthermore, proline application stimulates root nutrient absorption and enhances leaf mineral content, thereby optimizing photosynthetic efficiency and promoting favorable vegetative development [45].

Previous studies have established that exogenous proline preserves cellular osmotic equilibrium and mitigates ion toxicity by facilitating the sequestration or exclusion of excess Na⁺ and Cl⁻ ions [46]. Superoxide dismutase serves as a critical first-line defense component within the plant antioxidant arsenal, rapidly converting superoxide ions into H₂O₂ and O₂ [47]. Peroxidase, in turn, plays a predominant role in the efficient elimination of H₂O₂ from cellular compartments. The present study revealed that proline application significantly enhanced SOD, POD, and CAT activities, thereby accelerating ROS scavenging and reducing peroxidative membrane damage. These results corroborate the findings of [44], who reported that proline mitigates oxidative stress by activating antioxidant enzyme systems and exerting direct antioxidant effects. Collectively, the data presented herein indicate that exogenous proline application promotes lettuce growth and productivity under saline conditions through the preservation of photosynthetic pigment integrity and the enhancement of antioxidant enzyme accumulation, thereby establishing a robust defense against salinity-induced oxidative stress.

Conclusions

Salinity stress (50 mM NaCl) significantly inhibited lettuce growth, photosynthetic pigments, and mineral ion balance while inducing oxidative stress. Foliar application of proline at 200 mg/L effectively alleviated these adverse effects by enhancing vegetative growth, preserving chlorophyll and carotenoid contents, restoring K⁺/Na⁺ homeostasis, and upregulating antioxidant enzyme activities (POD, SOD, and CAT). These findings indicate that exogenous proline application represents a promising, sustainable strategy for improving lettuce productivity under saline conditions.

Conflict of interest. Nil

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