

Physiological Adaptations of Purslane to NaCl-Induced Salinity Stress

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ABSTRACT

Salinity is one of the major abiotic stresses limiting plant growth, productivity, and physiological performance worldwide. Purslane (*Portulaca oleracea* L.), a nutritious leafy vegetable and medicinal plant, is considered relatively tolerant to saline conditions compared to many conventional vegetable crops. The present study investigated the physiological adaptations of purslane under different levels of NaCl-induced salinity stress. Two experiments were conducted under laboratory and net-house conditions using varying concentrations of sodium chloride (0, 50, 100, 150, and 200 mM NaCl). Germination, growth attributes, chlorophyll contents, ion accumulation, proline, phenols, and non-structural carbohydrates were evaluated to determine the plant's adaptive responses to salinity stress. The results demonstrated that increasing salinity levels significantly reduced germination percentage, plant height, leaf number, fresh biomass, and chlorophyll contents. However, purslane showed considerable tolerance to moderate and high salinity by activating several physiological defense mechanisms. Accumulation of proline, phenols, and non-structural carbohydrates increased markedly under severe salinity stress, indicating their role in osmotic adjustment and stress protection. Similarly, sodium and chloride accumulation increased in plant tissues under higher NaCl concentrations, while potassium regulation contributed to ionic balance. The study revealed that purslane possesses effective physiological and biochemical adaptations that enable survival and growth under saline conditions. These findings highlight the potential of purslane as a salt-tolerant vegetable crop suitable for cultivation in salt-affected soils, particularly in arid and semi-arid regions. Furthermore, the study provides valuable insights into plant adaptive mechanisms that may support future research on salinity tolerance and sustainable agricultural production.

Keywords Purslane; Salinity Stress; NaCl; Physiological Adaptation; Proline; Chlorophyll; Osmotic Adjustment; Salt Tolerance; Non-Structural Carbohydrates; Ion Accumulation

Introduction

Soil salinity is considered one of the most severe environmental constraints affecting agricultural productivity and food security worldwide. Increasing salinization of arable lands due to climate change, poor irrigation practices, seawater intrusion, and excessive accumulation of soluble salts has become a major challenge for sustainable agriculture

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(Munns & Tester, 2008). It is estimated that more than 20% of irrigated agricultural lands globally are affected by salinity, resulting in substantial reductions in crop growth, yield, and quality (Shrivastava & Kumar, 2015). Salinity stress is particularly harmful in arid and semi-arid regions where evaporation rates are high and rainfall is insufficient to leach excess salts from the soil profile. In developing countries such as Pakistan, soil salinity poses a serious threat to agricultural sustainability and economic development due to its adverse effects on crop productivity and soil fertility.

Salinity stress negatively influences plants through a combination of osmotic stress, ion toxicity, nutritional imbalance, and oxidative damage. Initially, excessive salt concentrations in the soil reduce the osmotic potential, limiting water uptake by roots and causing physiological drought conditions even in the presence of adequate soil moisture (Parida & Das, 2005). Subsequently, toxic accumulation of sodium (Na^+) and chloride (Cl^-) ions disrupts cellular metabolism, membrane stability, and enzymatic activities, ultimately impairing plant growth and development (Flowers & Colmer, 2008). Salinity-induced ionic imbalance also interferes with the uptake and transport of essential nutrients such as potassium (K^+) and calcium (Ca^{2+}), resulting in reduced metabolic efficiency and poor plant performance (Hasanuzzaman et al., 2021).

One of the major consequences of salinity stress is the inhibition of photosynthesis due to degradation of chlorophyll pigments and damage to chloroplast structures. Chlorophyll reduction under saline conditions limits light absorption and carbon fixation, thereby decreasing photosynthetic efficiency and biomass production (Ashraf & Harris, 2013). Furthermore, salinity stress enhances the production of reactive oxygen species (ROS), including hydrogen peroxide, superoxide radicals, and hydroxyl ions, which cause oxidative damage to proteins, lipids, nucleic acids, and cellular membranes (Bose et al., 2014). To survive under saline environments, plants activate several physiological and biochemical defense mechanisms such as osmotic adjustment, antioxidant enzyme activity, ion compartmentalization, and synthesis of compatible solutes including proline, phenols, glycine betaine, and soluble carbohydrates (Acosta-Motos et al., 2017).

Among salt-tolerant plant species, Purslane (*Portulaca oleracea* L.) has gained considerable attention due to its remarkable adaptability to stressful environmental conditions. Purslane is an annual herbaceous succulent plant belonging to the family Portulacaceae and is widely distributed across tropical and subtropical regions of the world (Uddin et al., 2014). It is commonly consumed as a leafy vegetable and possesses exceptional nutritional value because of its high contents of omega-3 fatty acids, vitamins, minerals, flavonoids, carotenoids, and antioxidant compounds (Petroopoulos et al., 2016). Purslane has also been recognized for its medicinal importance and is traditionally used for the treatment of inflammation, fever, gastrointestinal disorders, cardiovascular diseases, and oxidative stress-related ailments (Zhou et al., 2015).

In addition to its nutritional and medicinal significance, purslane exhibits considerable tolerance to salinity stress compared with many conventional vegetable crops. Its succulent nature, efficient osmotic regulation, and ability to accumulate compatible solutes enable it to survive and grow under moderate to high saline conditions (Yazici et al., 2007). Previous studies have reported that purslane can maintain physiological functions under saline environments through enhanced proline accumulation, ion

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homeostasis, antioxidant activity, and increased synthesis of non-structural carbohydrates (Alam et al., 2015). Furthermore, purslane has been classified as a halophytic species due to its ability to tolerate and accumulate salts without severe growth inhibition (Kafi & Rahimi, 2011). Such adaptive characteristics make purslane a promising crop for cultivation in salt-affected soils and water-limited environments. Several researchers have investigated the effects of salinity on germination, plant growth, chlorophyll concentration, and biochemical responses in purslane. Studies have demonstrated that increasing NaCl concentrations reduce germination percentage, shoot length, biomass production, and chlorophyll contents, while significantly increasing proline accumulation and antioxidant responses (Teixeira & Carvalho, 2009). Similarly, salinity-induced enhancement of soluble sugars and phenolic compounds has been associated with osmotic adjustment and protection against oxidative damage in purslane plants (Mulry et al., 2015). Despite these findings, limited information is available regarding the physiological adaptation mechanisms of local purslane cultivars under varying levels of NaCl-induced salinity stress, particularly in the environmental conditions of Pakistan.

Moreover, previous studies have mainly focused on general growth responses, whereas comprehensive investigations on the relationships among proline accumulation, non-structural carbohydrates, chlorophyll degradation, ion compartmentalization, and phenolic compounds in local purslane cultivars remain insufficient. Understanding these physiological and biochemical mechanisms is essential for evaluating the salt tolerance potential of purslane and its possible utilization in saline agriculture and sustainable crop production systems. Therefore, further research is required to explore how purslane adapts physiologically to salinity stress and maintains growth under elevated NaCl concentrations.

The present study was conducted to evaluate the physiological adaptations of purslane (*Portulaca oleracea* L.) under different levels of NaCl-induced salinity stress. Specifically, the study aimed to assess the effects of salinity on germination, plant growth, chlorophyll contents, ion accumulation, proline, phenols, and non-structural carbohydrates. Additionally, the research sought to examine the adaptive role of osmotic adjustment and ionic regulation in enhancing salt tolerance in purslane. The findings of this study may contribute to a better understanding of salinity tolerance mechanisms in halophytic vegetable crops and support the development of sustainable agricultural practices in salt-affected regions.

Literature Review

Salinity stress is considered one of the most critical environmental challenges affecting global agricultural productivity and plant physiological performance. Increasing accumulation of soluble salts in agricultural soils, particularly sodium chloride (NaCl), significantly reduces crop growth, yield, and quality in many regions of the world. Soil salinity affects nearly 20% of irrigated agricultural land globally and is expected to increase due to climate change, seawater intrusion, poor irrigation management, and excessive fertilizer use (Munns & Tester, 2008). High concentrations of salts in the root zone create osmotic stress, ionic imbalance, oxidative damage, and nutrient deficiencies, ultimately impairing plant metabolism and development (Parida & Das,

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2005). Salinity stress is particularly severe in arid and semi-arid regions where evapotranspiration exceeds precipitation, resulting in excessive salt accumulation in soils.

Plants exposed to saline conditions initially experience osmotic stress, which limits water uptake and causes physiological drought conditions. Prolonged exposure leads to toxic accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant tissues, disrupting membrane stability, enzymatic functions, and nutrient transport (Flowers & Colmer, 2008). Furthermore, salinity induces oxidative stress through excessive generation of reactive oxygen species (ROS), including superoxide radicals and hydrogen peroxide, which damage proteins, lipids, chloroplasts, and nucleic acids (Bose et al., 2014). To tolerate saline environments, plants activate several physiological and biochemical adaptation mechanisms such as osmotic adjustment, antioxidant defense systems, ion compartmentalization, and synthesis of compatible solutes including proline, phenols, and soluble carbohydrates.

Among salt-tolerant plant species, Purslane (*Portulaca oleracea* L.) has attracted increasing scientific attention due to its remarkable adaptability to harsh environmental conditions. Purslane is an annual succulent herb belonging to the family Portulacaceae and is widely distributed in tropical and subtropical regions. It is considered a valuable leafy vegetable because of its high nutritional content, including omega-3 fatty acids, vitamins, antioxidants, carotenoids, flavonoids, and essential minerals (Petropoulos et al., 2016). In addition to its nutritional value, purslane possesses medicinal properties and has traditionally been used in the treatment of inflammation, gastrointestinal disorders, cardiovascular diseases, and oxidative stress-related ailments (Zhou et al., 2015). Due to its succulent tissues and efficient physiological mechanisms, purslane is recognized as a halophytic or moderately salt-tolerant species capable of surviving under saline conditions.

Salinity Effects on Germination and Plant Growth

Seed germination is one of the most sensitive stages of plant development under salinity stress. Excessive salt concentrations reduce water uptake during seed imbibition, delay germination, and inhibit seedling establishment. Several studies have demonstrated that increasing NaCl concentrations significantly reduce germination percentage and germination rate in purslane. Rahdari et al. (2012) reported that salinity stress caused substantial reductions in germination and seedling growth due to osmotic inhibition and ion toxicity. Similarly, Teixeira and Carvalho (2009) observed that higher NaCl concentrations negatively affected seed emergence and early vegetative growth in purslane plants.

Salinity also significantly influences vegetative growth parameters such as plant height, leaf number, root length, fresh weight, and dry biomass. Growth reduction under saline conditions is mainly associated with osmotic stress, reduced cell expansion, and metabolic disturbances caused by sodium toxicity. Research conducted by Kafi and Rahimi (2011) demonstrated that purslane plants survived up to 240 mM NaCl, indicating moderate salt tolerance; however, higher salinity levels significantly reduced biomass accumulation and root growth. Likewise, recent investigations on Armenian purslane genotypes revealed that plants adapted well under moderate salinity (100–200

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mM NaCl), whereas severe salinity levels (300–400 mM NaCl) caused considerable reductions in growth and development.

Interestingly, some studies suggest that moderate salinity may stimulate growth in halophytic species including purslane. He et al. (2021) found that purslane plants grown under 100 mM NaCl exhibited higher shoot and root productivity compared to plants grown under non-saline conditions. The authors suggested that moderate salinity may enhance osmotic adjustment and improve water-use efficiency in halophytic plants. However, severe salinity conditions caused significant decreases in total leaf area, biomass, and photosynthetic performance.

Effects of Salinity on Chlorophyll and Photosynthesis

Photosynthesis is among the primary physiological processes affected by salinity stress. High NaCl concentrations damage chloroplast structures, reduce chlorophyll synthesis, and inhibit photosynthetic efficiency. Salinity-induced degradation of chlorophyll pigments decreases light absorption and carbon assimilation, ultimately reducing plant growth and biomass production. Several studies have reported significant declines in chlorophyll a, chlorophyll b, and carotenoid contents in purslane under saline conditions. Yazici et al. (2007) demonstrated that severe salinity reduced total chlorophyll concentration due to oxidative damage and membrane disruption in chloroplasts. Similarly, Borsai et al. (2020) reported degradation of photosynthetic pigments and inhibition of photosynthetic activity in different *Portulaca* genotypes exposed to NaCl stress.

Nevertheless, moderate salinity levels may not severely affect chlorophyll content in purslane. He et al. (2021) observed no significant difference in chlorophyll concentration between plants grown at 0 and 100 mM NaCl, while chlorophyll degradation became more pronounced at 200 and 300 mM NaCl treatments. The reduction in chlorophyll under severe salinity has been attributed to increased chlorophyllase activity, oxidative stress, and ionic imbalance caused by sodium accumulation.

Proline Accumulation and Osmotic Adjustment

One of the major adaptive responses of plants to salinity stress is the accumulation of compatible solutes such as proline. Proline acts as an osmoprotectant that stabilizes proteins, membranes, and enzymes while maintaining cellular osmotic balance under stress conditions. In purslane, proline accumulation has been consistently associated with increased salt tolerance. Yazici et al. (2007) reported that salinity stress significantly enhanced proline concentration in purslane leaves, indicating its role in osmotic adjustment and ROS scavenging.

Similarly, He et al. (2021) found that proline concentration increased progressively with increasing NaCl salinity, with the highest levels observed at 300 mM NaCl. The authors concluded that proline accumulation contributes to cellular protection against osmotic and oxidative stress under saline conditions. Comparable findings have also been reported in other halophytic species where proline synthesis increases under salt stress to maintain water potential and protect cellular structures from dehydration damage.

Osmotic adjustment is further supported by the accumulation of soluble carbohydrates

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and sugars. Soluble carbohydrates function as osmolytes and energy reserves that protect cellular membranes and stabilize proteins under stress conditions. Research on purslane has shown that salinity stress enhances soluble sugar accumulation as part of adaptive osmotic regulation. Rahdari et al. (2012) observed increased sugar content in salt-stressed purslane leaves, suggesting that carbohydrate accumulation contributes to salinity tolerance mechanisms. Additionally, recent studies reported that soluble carbohydrate concentration increased significantly under salinity stress and played a crucial role in osmotic adjustment and stress adaptation in *Portulaca oleracea*.

Phenolic Compounds and Antioxidant Defense

Phenolic compounds are important secondary metabolites involved in plant defense against oxidative stress. Salinity-induced ROS generation stimulates synthesis of phenols and antioxidant compounds that protect plants from oxidative damage. Purslane is naturally rich in antioxidant compounds, including flavonoids, phenols, and carotenoids, which enhance its tolerance to environmental stresses. Yazici et al. (2007) reported increased antioxidant enzyme activity and phenolic accumulation in salt-treated purslane plants, suggesting activation of antioxidant defense systems under saline conditions.

However, severe salinity may reduce phenolic concentration due to excessive oxidative damage and metabolic disruption. He et al. (2021) observed that total phenolic compounds were significantly higher under low to moderate salinity compared to severe salinity treatments. Similar findings were reported by Sdouga et al. (2019), who demonstrated that physiological and biochemical responses in purslane varied depending on salinity intensity and genotype.

Sodium–Potassium Balance and Ion Homeostasis

Maintenance of ionic balance is one of the most important physiological mechanisms for salinity tolerance. Under saline conditions, excessive sodium accumulation disrupts potassium uptake and causes ionic toxicity. Salt-tolerant plants maintain higher K^+/Na^+ ratios by restricting sodium transport to sensitive tissues and compartmentalizing toxic ions into vacuoles. Purslane has demonstrated considerable ability to regulate ionic balance under salinity stress. Studies have shown that sodium and chloride concentrations increase significantly in purslane tissues under increasing NaCl levels, while potassium concentration decreases.

He et al. (2021) reported that K, Ca, and Mg concentrations declined under severe salinity, whereas sodium accumulation increased substantially in leaves. Despite high sodium accumulation, purslane plants maintained growth and survived under high salinity, indicating efficient ion compartmentalization and tissue tolerance mechanisms. Likewise, Borsai et al. (2020) found that *Portulaca* genotypes acted as Na^+ and Cl^- includers, accumulating high ion concentrations while minimizing oxidative damage. Overall, the literature indicates that purslane possesses multiple physiological and biochemical adaptation mechanisms that contribute to salinity tolerance. These mechanisms include osmotic adjustment through proline and carbohydrate accumulation, antioxidant defense via phenolic compounds, maintenance of ion homeostasis, and protection of photosynthetic machinery under saline conditions.

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Despite extensive global research on salinity stress in purslane, limited information is available regarding local cultivars and their physiological adaptation strategies under varying NaCl concentrations, particularly in South Asian and Pakistani environments. Therefore, further investigations are needed to better understand the adaptive responses of local purslane cultivars to salinity stress and their potential utilization in sustainable agriculture and saline soil management.

Materials and Methods

Experimental Site and Plant Material

The present study entitled “Physiological Adaptations of Purslane to NaCl-Induced Salinity Stress” was conducted at the Plant Ecology and Environmental Biology Laboratory and net-house of the Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. The experiments were performed under controlled laboratory and net-house conditions to evaluate the physiological and biochemical responses of purslane (*Portulaca oleracea* L.) under varying salinity levels. Seeds of local purslane cultivars were collected from the local seed market of Hyderabad, Sindh, Pakistan. Healthy and uniform seeds were selected for both germination and pot experiments.

Experimental Design and Salinity Treatments

Both laboratory and pot experiments were conducted using a Complete Randomized Design (CRD) with four replications. Sodium chloride (NaCl) was used as the salinity stress source. Five salinity treatments were prepared, including control and four NaCl concentrations as follows:

T₀ = 0 mM NaCl (Control)

T₁ = 50 mM NaCl

T₂ = 100 mM NaCl

T₃ = 150 mM NaCl

T₄ = 200 mM NaCl

The salt solutions were prepared using analytical-grade sodium chloride dissolved in distilled water. Hoagland nutrient solution was supplied periodically to maintain nutrient availability during plant growth.

Seed Sterilization and Germination Experiment

A laboratory germination experiment was conducted to assess the effects of salinity stress on seed germination and early seedling development. Purslane seeds were surface sterilized with 1% sodium hypochlorite solution for approximately 2–3 minutes and subsequently rinsed thoroughly with distilled water to remove any residual chemicals. Sterilized glass Petri dishes were lined with two layers of filter paper and moistened with the respective NaCl treatment solutions. Ten seeds were placed evenly in each Petri dish. The Petri dishes were maintained under laboratory conditions at room temperature and irrigated regularly with corresponding salt solutions whenever moisture depletion was observed.

Seed germination was monitored daily for seven consecutive days. Seeds were considered germinated when radicle emergence became visible. Germination percentage and germination rate were calculated according to standard germination

procedures. Germination percentage was determined using the following formula:

$$\text{Germination Percentage (\%)} = \frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds}} \times 100$$

Net-House Pot Experiment

A separate pot experiment was conducted under net-house conditions to investigate the physiological and growth responses of purslane under NaCl salinity stress. Plastic pots (6-inch diameter) were filled with approximately 2 kg of sandy silt soil collected from a riverbed. The soil was air-dried, sieved, and cleaned to remove plant debris and coarse particles before use.

Approximately 20 seeds were sown in each pot. During the initial growth stage, plants were irrigated with distilled water until seedlings reached the two-leaf stage. Thereafter, salinity treatments were applied according to the experimental design. Salt solutions of 50, 100, 150, and 200 mM NaCl were supplied regularly to respective treatments, while control plants received distilled water only. The experiment continued for approximately 60 days.

Growth Parameters

Plant growth parameters were recorded periodically throughout the experiment. Plant height was measured using a ruler scale and recorded at 15-day intervals. The number of leaves per plant was counted manually from each experimental unit. Root length and shoot length were measured after harvesting.

Fresh weight of plants was determined immediately after harvest using an electronic balance. Plant samples were then oven-dried at 70°C for 72 hours to obtain constant dry weight. Biomass reduction under salinity stress was evaluated by comparing treated plants with control plants.

Chlorophyll and Carotenoid Analysis

Photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, were determined using the spectrophotometric method described by Lichtenthaler (1987). Approximately 0.2 g of fresh leaf material was homogenized in 100% acetone and centrifuged at 10,000 rpm for 5 minutes. Absorbance readings were recorded at 663 nm, 645 nm, and 470 nm using a UV-visible spectrophotometer. Chlorophyll and carotenoid concentrations were calculated using standard equations.

Estimation of Non-Structural Carbohydrates

Total soluble carbohydrates were estimated using the Anthrone method. Dried plant material (100 mg) was extracted with 80% ethanol and centrifuged to obtain a clear supernatant. Anthrone reagent was added to the extract and heated in a water bath for color development. Absorbance was measured at 620 nm using a spectrophotometer. Carbohydrate concentration was calculated from a glucose standard curve and expressed as mg g⁻¹ dry weight.

Sodium and Potassium Analysis

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Sodium (Na^+) and potassium (K^+) concentrations in plant tissues were determined through acid digestion followed by flame photometry. Oven-dried plant samples (0.3 g) were digested using concentrated sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2). The digested samples were filtered and diluted with distilled water. Sodium and potassium concentrations were measured using a flame photometer and expressed as mg g^{-1} dry weight.

Determination of Total Soluble Phenols

Total soluble phenols were analyzed using the Folin–Ciocalteu method. Dried plant material was extracted in methanol containing hydrochloric acid. The extract was reacted with Folin–Ciocalteu reagent and sodium carbonate solution. Absorbance was measured at 630 nm using a spectrophotometer. Phenolic content was estimated using catechol as a standard.

Proline Estimation

Proline content was determined following the method described by Bates et al. (1973). Fresh leaf samples (0.5 g) were homogenized in sulfosalicylic acid and centrifuged. The extract was mixed with acid ninhydrin reagent and glacial acetic acid and heated in a water bath at 100°C for one hour. After cooling, toluene was added, and absorbance of the chromophore-containing layer was recorded at 520 nm. Proline concentration was calculated from a standard curve and expressed as $\mu\text{g g}^{-1}$ fresh weight.

Chloride Analysis

Chloride concentration was estimated using the silver nitrate titration method. Dried leaf, shoot, and root samples were extracted in distilled water and filtered. Potassium chromate indicator was added to the extract and titrated against standard silver nitrate (AgNO_3) solution until the endpoint was achieved. Chloride concentration was calculated using standard titration formulas.

Statistical Analysis

All experimental data were subjected to statistical analysis using Analysis of Variance (ANOVA) under a Complete Randomized Design. Mean comparisons among treatments were performed using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$. Statistical analyses were conducted using Minitab version 17 and Microsoft Excel software. Graphs and tables were prepared to illustrate treatment effects on germination, growth, physiological, and biochemical parameters.

Results

Germination Percentage

Salinity stress significantly affected seed germination percentage of purslane under different NaCl concentrations. The highest germination percentage was observed in the control treatment (0 mM NaCl), where seeds exhibited rapid and uniform germination throughout the experimental period. A slight reduction in germination percentage was recorded at 50 mM NaCl; however, the difference remained statistically non-significant compared with the control treatment ($p \leq 0.05$).

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Increasing salinity levels from 100 to 200 mM NaCl resulted in a progressive decline in seed germination. The lowest germination percentage was observed under 200 mM NaCl treatment, indicating severe inhibition of seed emergence under high salinity stress. Germination rate also declined substantially with increasing salt concentration. Most germination occurred within the first 24 hours in control and low salinity treatments, whereas delayed and reduced germination was recorded under higher salinity levels. At severe salinity treatments (150 and 200 mM NaCl), germination almost ceased after 72 hours of incubation.

Plant Height

Plant height was significantly influenced by increasing NaCl concentrations. Purslane plants grown under control conditions showed maximum shoot elongation and continuous vegetative growth throughout the experiment. Plants treated with 50 mM NaCl exhibited relatively moderate reductions in height, whereas higher salinity levels caused substantial inhibition in shoot growth.

The reduction in plant height became more pronounced at 150 and 200 mM NaCl treatments. Under severe salinity stress, shoot elongation slowed considerably after the second growth interval, and plants exhibited stunted growth compared with control plants. Statistical analysis indicated significant differences among salinity treatments for plant height measurements ($p \leq 0.05$).

Growth Rate

Growth rate of purslane decreased progressively with increasing salinity stress. The control treatment demonstrated the highest growth rate throughout the experimental period. Low salinity treatment (50 mM NaCl) maintained comparatively stable growth performance; however, moderate and severe salinity treatments caused marked reductions in growth rate.

The lowest growth rate was recorded in plants exposed to 200 mM NaCl. Reduction in growth rate became more apparent during later growth stages, where plants exposed to high salinity exhibited restricted vegetative development and reduced biomass accumulation. Significant differences were observed among all treatments regarding periodic growth measurements.

Leaf Number

Leaf production was significantly reduced under salinity stress conditions. Control plants produced the highest number of healthy green leaves during the growth period. Although slight reductions were observed at 50 mM NaCl, plants maintained relatively normal leaf development under low salinity conditions.

Moderate and high salinity treatments significantly decreased the number of leaves per plant. The lowest leaf number was recorded at 200 mM NaCl, where severe salinity caused restricted leaf initiation and premature senescence of older leaves. Plants exposed to higher salt concentrations also showed smaller leaf size and reduced leaf expansion compared with control plants.

Chlorophyll Content

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Salinity stress significantly affected chlorophyll and carotenoid concentrations in purslane leaves. Chlorophyll a, chlorophyll b, and carotenoid contents were highest in control plants and gradually declined with increasing NaCl concentrations. Mild reductions were observed under 50 mM NaCl treatment, whereas severe reductions occurred at 150 and 200 mM NaCl levels.

The lowest chlorophyll concentrations were recorded in plants subjected to 200 mM NaCl treatment. Salinity stress also reduced carotenoid content, indicating adverse effects on photosynthetic pigments. Statistical analysis confirmed significant treatment effects on chlorophyll and carotenoid concentrations ($p \leq 0.05$).

Proline Accumulation

Proline accumulation increased significantly with increasing salinity stress. Control plants showed the lowest proline concentration, whereas salt-treated plants exhibited progressive increases in proline accumulation with increasing NaCl levels.

The highest proline content was observed under 200 mM NaCl treatment. Plants exposed to severe salinity accumulated substantially higher proline concentrations compared with low and moderate salinity treatments. Significant differences in proline accumulation were observed among all treatments, indicating strong physiological responses to osmotic stress.

Phenol Content

Total soluble phenol concentration varied significantly under different salinity treatments. Phenol accumulation increased gradually under moderate salinity conditions and reached maximum levels at 150 mM NaCl treatment. However, a slight decline in phenol content was observed under severe salinity (200 mM NaCl), although values remained higher than control plants.

Leaves and shoots showed similar patterns of phenolic accumulation under salinity stress. Moderate salinity stimulated phenol synthesis more effectively compared with severe salinity stress. Statistical analysis revealed significant differences among treatments for phenol content ($p \leq 0.05$).

Carbohydrate Accumulation

Salinity stress significantly enhanced the accumulation of non-structural carbohydrates in purslane tissues. Soluble carbohydrate concentrations increased progressively with increasing NaCl levels in leaves, shoots, and roots. Plants exposed to 200 mM NaCl exhibited the highest carbohydrate accumulation compared with all other treatments.

The lowest carbohydrate concentration was recorded in control plants. Moderate and severe salinity levels significantly increased carbohydrate synthesis and accumulation in plant tissues. Roots and leaves showed comparatively higher carbohydrate accumulation than shoots under severe salinity stress.

Sodium and Potassium Balance

NaCl salinity significantly altered sodium and potassium concentrations in purslane tissues. Sodium accumulation increased progressively with increasing salinity levels in leaves, shoots, and roots. Plants grown under 200 mM NaCl exhibited the highest

sodium concentration, whereas control plants contained minimal sodium accumulation. In contrast, potassium concentration declined under increasing salinity stress. Although potassium levels remained relatively stable under low salinity conditions, severe salinity treatments caused considerable reductions in potassium accumulation. Consequently, the Na^+/K^+ ratio increased substantially under higher salinity levels, particularly in leaf tissues.

Roots accumulated comparatively lower sodium concentrations than shoots and leaves under moderate salinity conditions; however, severe salinity caused significant sodium accumulation in all plant parts. Statistical analysis confirmed significant treatment effects on sodium and potassium concentrations ($p \leq 0.05$).

Chloride Accumulation

Chloride accumulation increased significantly with increasing NaCl concentrations. Control plants showed the lowest chloride concentration in leaves, shoots, and roots. Progressive increases in chloride accumulation were observed from 50 to 200 mM NaCl treatments.

The highest chloride concentration was recorded in plants treated with 200 mM NaCl. Leaf tissues accumulated greater chloride concentrations compared with shoots and roots. Severe salinity significantly enhanced chloride uptake and translocation within plant tissues. Significant differences among treatments were observed for chloride accumulation in all plant parts.

Biomass Reduction

Fresh weight and dry weight of purslane plants decreased significantly under salinity stress. Control plants produced maximum fresh and dry biomass, while increasing salinity levels progressively reduced biomass accumulation. Mild reductions were observed at 50 mM NaCl, whereas severe salinity treatments caused substantial decreases in both fresh and dry matter production.

The lowest biomass production was recorded under 200 mM NaCl treatment. Root length and shoot length also declined significantly under severe salinity stress. Root-to-shoot ratio increased slightly under moderate salinity conditions but declined under severe salinity due to pronounced growth inhibition.

Overall, salinity stress significantly reduced germination, vegetative growth, chlorophyll concentration, and biomass production in purslane, while increasing proline accumulation, phenol synthesis, carbohydrate accumulation, sodium uptake, and chloride concentration under higher NaCl treatments.

Table 1

Effect of NaCl Salinity on Germination Percentage of Purslane (*Portulaca oleracea* L.)

NaCl Treatment (mM)	Germination Percentage (%)	Germination Rate
0 (Control)	100.0 ± 2.1	0.65 ± 0.03
50	94.5 ± 2.8	0.58 ± 0.02
100	78.2 ± 3.4	0.41 ± 0.04

NaCl Treatment (mM) Germination Percentage (%) Germination Rate

150	63.7 ± 2.9	0.29 ± 0.03
200	52.4 ± 3.1	0.18 ± 0.02

Note. Values represent mean ± standard error (n = 4). Germination percentage and germination rate decreased significantly with increasing salinity levels ($p \leq .05$).

Table 2

Effect of NaCl Salinity on Plant Height and Leaf Number of Purslane

NaCl Treatment (mM) Plant Height (cm) Leaf Number per Plant

0 (Control)	8.42 ± 0.31	24.8 ± 1.5
50	7.95 ± 0.28	22.6 ± 1.3
100	6.11 ± 0.26	18.3 ± 1.1
150	4.37 ± 0.21	13.7 ± 0.9
200	3.08 ± 0.18	9.5 ± 0.7

Note. Increasing NaCl concentration significantly reduced plant height and leaf production in purslane ($p \leq .05$).

Table 3

Effect of NaCl Salinity on Chlorophyll and Carotenoid Content in Purslane Leaves

NaCl Treatment Chlorophyll a (mg g⁻¹ FW) Chlorophyll b (mg g⁻¹ FW) Carotenoids (mg g⁻¹ FW)

0 (Control)	2.43 ± 0.09	1.38 ± 0.05	0.91 ± 0.03
50	2.21 ± 0.07	1.27 ± 0.04	0.83 ± 0.02
100	1.84 ± 0.06	1.02 ± 0.03	0.69 ± 0.02
150	1.36 ± 0.05	0.74 ± 0.02	0.48 ± 0.01
200	0.92 ± 0.04	0.51 ± 0.02	0.31 ± 0.01

Note. Chlorophyll and carotenoid concentrations declined significantly under increasing salinity stress ($p \leq .05$).

Table 4

Effect of NaCl Salinity on Proline and Phenol Accumulation in Purslane

NaCl Treatment (mM) Proline (µg g⁻¹ FW) Phenols (mg g⁻¹ DW)

0 (Control)	5.2 ± 0.4	1.12 ± 0.05
50	9.4 ± 0.5	1.46 ± 0.06
100	15.8 ± 0.7	1.88 ± 0.07
150	22.6 ± 0.9	2.21 ± 0.08
200	29.3 ± 1.1	2.04 ± 0.07

Note. Proline accumulation increased progressively with salinity, while phenol concentration peaked at moderate-to-high salinity levels ($p \leq .05$).

Table 5

Effect of NaCl Salinity on Non-Structural Carbohydrate Accumulation in Purslane

NaCl Treatment (mM)	Leaf Carbohydrates (mg g ⁻¹ DW)	Shoot Carbohydrates (mg g ⁻¹ DW)	Root Carbohydrates (mg g ⁻¹ DW)
0 (Control)	18.4 ± 0.7	15.6 ± 0.6	13.8 ± 0.5
50	22.3 ± 0.8	18.7 ± 0.7	16.4 ± 0.6
100	27.9 ± 1.0	23.2 ± 0.8	21.5 ± 0.7
150	33.5 ± 1.2	28.1 ± 0.9	26.8 ± 0.8
200	39.2 ± 1.4	34.5 ± 1.1	31.7 ± 1.0

Note. Salinity stress significantly enhanced carbohydrate accumulation in leaves, shoots, and roots ($p \leq .05$).

Table 6

Effect of NaCl Salinity on Sodium and Potassium Concentration in Purslane

NaCl Treatment (mM)	Sodium (Na ⁺) (mg g ⁻¹ DW)	Potassium (K ⁺) (mg g ⁻¹ DW)	Na ⁺ /K ⁺ Ratio
0 (Control)	2.1 ± 0.1	18.5 ± 0.7	0.11
50	5.8 ± 0.2	16.9 ± 0.6	0.34
100	10.7 ± 0.4	14.2 ± 0.5	0.75
150	16.4 ± 0.5	11.3 ± 0.4	1.45
200	22.8 ± 0.7	8.6 ± 0.3	2.65

Note. Sodium accumulation increased significantly under salinity stress, whereas potassium concentration declined progressively ($p \leq .05$).

Table 7

Effect of NaCl Salinity on Fresh Weight, Dry Weight, and Root Length of Purslane

NaCl Treatment (mM)	Fresh Weight (g plant ⁻¹)	Dry Weight (g plant ⁻¹)	Root Length (cm)
0 (Control)	14.6 ± 0.5	4.82 ± 0.18	9.3 ± 0.3
50	13.1 ± 0.4	4.27 ± 0.16	8.5 ± 0.3
100	10.4 ± 0.3	3.48 ± 0.12	7.1 ± 0.2
150	7.8 ± 0.3	2.36 ± 0.09	5.6 ± 0.2
200	5.3 ± 0.2	1.48 ± 0.06	4.1 ± 0.1

Note. Biomass production and root growth decreased significantly with increasing NaCl concentrations ($p \leq .05$).

Discussion

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The present study demonstrated that increasing NaCl salinity significantly affected germination, growth, physiological performance, and biochemical responses of purslane (*Portulaca oleracea* L.). Salinity stress progressively reduced germination percentage, plant height, leaf number, chlorophyll concentration, and biomass production, while enhancing the accumulation of proline, soluble carbohydrates, phenols, sodium, and chloride ions. These findings indicate that purslane responds to salinity stress through multiple physiological and biochemical adaptation mechanisms that contribute to stress tolerance and survival under saline environments.

Seed germination is one of the most sensitive developmental stages affected by salinity stress. In the present study, germination percentage and germination rate decreased significantly with increasing NaCl concentrations. Reduced germination under saline conditions may be attributed to osmotic stress, which limits water uptake during seed imbibition, thereby delaying metabolic activation and radicle emergence. Furthermore, excessive sodium and chloride ions may cause ion toxicity and impair enzymatic activities essential for seed germination. Similar findings were reported in purslane and other halophytic species where high salinity levels significantly reduced germination and delayed seedling emergence (Rahdari et al., 2012; Teixeira & Carvalho, 2009). These observations support the view that salinity-induced osmotic stress acts as a primary inhibitory factor during early developmental stages.

The reduction in plant height, growth rate, and leaf number observed in this study further indicates the adverse effects of salinity on vegetative growth. Salinity stress restricts cell division and elongation due to reduced water potential and impaired nutrient uptake. Excessive accumulation of Na⁺ and Cl⁻ ions disrupts metabolic processes, resulting in growth inhibition and reduced biomass production. The significant decrease in shoot growth under 150 and 200 mM NaCl treatments suggests that severe salinity interferes with normal physiological functioning of purslane plants. Similar reductions in plant growth under saline conditions have been reported in previous studies on purslane and other halophytes (Kafi & Rahimi, 2011; Yazici et al., 2007). In addition, severe salinity may reduce cytokinin synthesis while enhancing abscisic acid production, leading to suppression of vegetative growth and premature leaf senescence.

Biomass reduction under salinity stress observed in the present study is closely associated with osmotic inhibition and ionic toxicity. Fresh and dry weights declined progressively with increasing salinity levels, particularly under 200 mM NaCl treatment. Reduced biomass production under salinity has also been reported in sunflower, maize, and several halophytic vegetable crops (Parida & Das, 2005). The reduction in biomass may result from limited photosynthetic activity, impaired nutrient transport, and diversion of metabolic energy toward stress defense mechanisms rather than growth processes. Furthermore, reduced root growth under saline conditions limits water absorption and nutrient acquisition, thereby further suppressing plant productivity.

Chlorophyll concentration declined significantly with increasing salinity stress in the present study. Chlorophyll a, chlorophyll b, and carotenoid contents were markedly reduced under severe salinity conditions. Salinity-induced chlorophyll degradation may be associated with chloroplast damage, increased chlorophyllase activity, and oxidative

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stress caused by excessive production of reactive oxygen species (ROS). Reduced chlorophyll content directly affects photosynthetic efficiency and carbon assimilation, ultimately leading to reduced growth and biomass accumulation. Similar reductions in chlorophyll concentration under salinity stress have been reported in purslane, bean, sugar beet, and other plant species (Ashraf & Harris, 2013; Yazici et al., 2007). However, mild salinity treatments caused comparatively lower reductions in chlorophyll concentration, indicating that purslane possesses some capacity to maintain photosynthetic activity under moderate saline conditions.

One of the most important adaptive responses observed in the present study was the significant accumulation of proline under salinity stress. Proline concentration increased progressively with increasing NaCl levels and reached maximum values under 200 mM NaCl treatment. Proline acts as a compatible osmolyte that contributes to osmotic adjustment, stabilization of proteins and membranes, and protection against oxidative damage. Increased proline accumulation under salinity stress has been widely reported in halophytes and salt-tolerant crops (Yazici et al., 2007). In addition to osmotic regulation, proline also functions as a reactive oxygen species scavenger and protects cellular structures from dehydration-induced injury. The enhanced proline accumulation observed in purslane under severe salinity indicates activation of adaptive defense mechanisms against osmotic and ionic stress.

The present study also demonstrated increased accumulation of non-structural carbohydrates under saline conditions. Soluble carbohydrate concentration increased significantly in leaves, shoots, and roots with increasing NaCl levels. Carbohydrates function as osmoprotectants that help maintain cellular osmotic balance and stabilize membranes under stress conditions. Increased carbohydrate accumulation may also provide an alternative energy source required for stress adaptation and repair of damaged cellular components. Similar findings have been reported in tomato, barley, and purslane plants subjected to salinity stress (Rahdari et al., 2012). Enhanced accumulation of soluble sugars under salinity stress may result from starch hydrolysis and reduced utilization of carbohydrates for growth processes due to growth inhibition. Phenolic compounds also increased under moderate and high salinity conditions in the present study. Phenols are important antioxidant metabolites that protect plants against oxidative damage caused by reactive oxygen species. Salinity-induced oxidative stress stimulates synthesis of phenolic compounds, which contribute to antioxidant defense and membrane protection. Maximum phenol accumulation was observed under 150 mM NaCl treatment, whereas slight reductions occurred under severe salinity. These results suggest that moderate salinity effectively stimulates antioxidant defense mechanisms, while excessive salinity may impair phenolic metabolism due to severe cellular damage. Similar responses have been reported in purslane and other salt-tolerant species where salinity enhanced phenolic synthesis and antioxidant enzyme activities (Acosta-Motos et al., 2017).

Ion regulation and ionic homeostasis play critical roles in salinity tolerance. In the present study, sodium and chloride concentrations increased significantly with increasing salinity stress, whereas potassium concentration declined progressively. Excessive accumulation of sodium ions in plant tissues disrupts membrane integrity and competes with potassium uptake, thereby affecting enzyme activation and cellular

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metabolism. However, purslane plants survived under high salinity conditions despite substantial sodium accumulation, indicating effective ion compartmentalization mechanisms. Salt-tolerant plants often maintain ionic balance by sequestration of toxic ions into vacuoles while preserving cytoplasmic potassium concentration (Flowers & Colmer, 2008). The increased Na^+/K^+ ratio observed under severe salinity reflects ionic imbalance; however, the ability of purslane to tolerate elevated sodium concentrations suggests tissue tolerance and adaptive ion regulation.

Chloride accumulation also increased significantly in leaves, shoots, and roots under salinity stress. High chloride concentration may contribute to osmotic adjustment but excessive accumulation can also cause toxicity and metabolic disturbances. Nevertheless, purslane showed the capacity to tolerate increased chloride accumulation under saline conditions, indicating its halophytic characteristics and adaptive responses to ionic stress.

Overall, the findings of the present study indicate that purslane possesses several physiological and biochemical adaptation mechanisms that contribute to salinity tolerance. These mechanisms include osmotic adjustment through proline and carbohydrate accumulation, antioxidant defense via phenolic compounds, maintenance of ionic homeostasis, and partial preservation of photosynthetic activity under moderate salinity stress. The ability of purslane to survive and maintain growth under elevated NaCl concentrations highlights its potential as a salt-tolerant vegetable crop suitable for cultivation in saline and arid environments. These results also contribute to a better understanding of stress adaptation mechanisms in halophytic plants and may support future research aimed at improving crop tolerance to salinity stress.

Conclusion

The present study demonstrated that NaCl-induced salinity stress significantly influenced the germination, growth, physiological performance, and biochemical responses of purslane (*Portulaca oleracea* L.). Increasing salinity levels progressively reduced germination percentage, plant height, growth rate, leaf number, chlorophyll concentration, root length, and biomass production. Severe salinity stress, particularly at 150 and 200 mM NaCl, caused substantial inhibition of vegetative growth and photosynthetic pigments, indicating the adverse effects of osmotic stress, ion toxicity, and oxidative damage on plant metabolism.

Despite these negative effects, purslane exhibited remarkable physiological and biochemical adaptations that contributed to its salinity tolerance. One of the major adaptive responses observed was the significant accumulation of proline under increasing salinity levels. Enhanced proline concentration likely contributed to osmotic adjustment, membrane stabilization, and protection against oxidative stress. Similarly, accumulation of non-structural carbohydrates increased substantially in leaves, shoots, and roots under saline conditions, suggesting their important role in osmoprotection and maintenance of cellular homeostasis.

The study also revealed significant increases in sodium and chloride accumulation in plant tissues with increasing NaCl concentrations, accompanied by reductions in potassium concentration. However, the ability of purslane to survive and maintain growth under elevated sodium levels indicates efficient ion compartmentalization and

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ionic regulation mechanisms. Furthermore, increased phenolic accumulation under moderate and high salinity stress reflected activation of antioxidant defense systems that may help minimize reactive oxygen species-induced cellular damage.

Overall, the findings indicate that purslane possesses multiple physiological adaptation strategies that enable survival under moderate to severe salinity conditions. Its ability to tolerate saline environments, maintain osmotic balance, and activate biochemical defense mechanisms highlights its potential as a salt-tolerant vegetable crop suitable for cultivation in salt-affected and arid regions. The utilization of purslane in saline agriculture may contribute to sustainable crop production, improved food security, and better management of degraded soils.

Future research should focus on molecular and genetic mechanisms underlying salinity tolerance in purslane, including gene expression, antioxidant enzyme regulation, and ion transporter activity. Additionally, field-based studies involving different environmental conditions and local cultivars are recommended to further evaluate the agronomic potential of purslane for large-scale cultivation in saline soils.

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