PREPRINT

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Seawater-induced Salinity Enhances Antioxidant Capacity by Modulating Morpho-physiological and Biochemical Responses in *Catharanthus roseus*

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ABSTRACT

Salt stress impedes plant growth and development due to several factors, including the generation of cellular oxidative stressors. This study aimed to assess the impacts of seawater-induced salinity on the plant development, physio-biochemical responses, and antioxidant capacity of *Catharanthus roseus* grown in a variety of seawater (4, 8, and 12 dS/m) for varying durations (60, 90, and 120 days). The experiment was laid out in a randomized complete block design with five replications. The results demonstrated that *C. roseus* successfully endured moderate salinity (8 dS/m) by maintaining plant height, number of leaves, branches, relative water content, and chlorophyll content with a minimum drop in dry biomass (25%) in a time- and dose-dependent approach. Furthermore, greater proline and soluble sugar contents suggested that *C. roseus* possessed enhanced osmoprotective capabilities to counteract osmotic stress caused by salinity. Conversely, all growth indicators decreased significantly at high salinity (12 dS/m). Increased levels of antioxidant enzyme activity catalase and ascorbate peroxidase, phenol and flavonoid, 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid indicate a coordinated function for antioxidant components in regulating reactive oxygen species (ROS) at low (4 dS/m) and moderate (8 dS/m) salinities. In contrast, excessive salinity

ARTICLE INFO

Article history:
Received: 20 December 2023
Accepted: 24 April 2024
Published: 30 October 2024

DOI: https://doi.org/10.47836/pjtas.47.4.13

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ISSN: 1511-3701
e-ISSN: 2231-8542

(12 dS/m) led to a burst of ROS, as seen by elevated levels of hydrogen peroxide, malondialdehyde, and electrolyte leakage that greatly reduced total dry matter (72%), especially on days 120. The ion studies on plants subjected to salinity revealed that most Na^+ remained in the roots. In contrast, most K^+, Ca^{2+}, and Mg^{2+} are deposited more firmly in the leaves than in the roots. The findings imply that *C. roseus* may tolerate moderate salinity (8 dS/m) owing to its enhanced antioxidant defense system and osmolytes, which trigger antioxidant enzymes and maintain ionic balance.

Keywords: Antioxidant, *Catharanthus roseus*, oxidative stress, proline, seawater

INTRODUCTION

Salinity is a substantial environmental constraint that significantly affects crop productivity and growth. The continued increase in salinity in arable land brought on by inadequate agricultural methods and climate change is predicted to result in the loss of half of the crop area by the middle of the twenty-first century. According to Akter et al. (2023), the amount of land in Bangladesh impacted by soil salinity continuously grows due to global warming, with a 26.73% increase in salt-prone areas between 1973 and 2009. Around 25–30% of Bangladesh's total arable land is in its salty regions.

Stress due to salt in plants has multitudes of adverse effects, including morpho-physiological, biochemical, and genetic alternations. Osmotic damage and toxicity from ions are the two primary ways that salinity impacts the development and growth of plants (Ali et al., 2022). Osmotic stress brought on by an excessive amount of salt in the root region impairs a root's capacity to absorb water because plant cells are limited in their ability to obtain water by a root signal mediated by abscisic acid (ABA). Secondly, a high salt content within the plant generates ionic toxicity (hyperosmotic stress), which results in cell death — additionally, increased Na^+ influx and K^+ efflux cause an increased Na^+/K^+ ratio in cell membranes, which causes plants to experience osmotic stress (Munns & Tester, 2008). Numerous physiological and biochemical processes, including nutrient intake (such as K^+, Ca^{2+}, and Mg^{2+}) and CO_2 assimilation, can be impaired by high salt concentrations, notably higher Na^+ concentrations in the transpiration stream of plants (Sarker et al., 2018). The formation of harmful ions modifies how plants interact with water and hinders their photosynthetic pigments, which causes a decrease in transpiration rates, growth, photosynthesis, and biomass production (Dawood et al., 2014).

All these physiological modifications caused by salt in plants exacerbate the overabundance of reactive oxygen species (ROS), which interferes with the regular metabolism of cells and causes oxidative damage by oxidizing lipids, proteins, DNA, and other macromolecules within cells (A. K. Das et al., 2022). To counteract salinity's detrimental effects, plants display various morpho-physiological and molecular

adaptations (Rahman et al., 2017). Plants can generate osmolytes such as soluble sugars, amino acids, and proline (Pro) that shield plant cells from the damaging effects of salt exposure (Sarker et al., 2018). These facilitate adjustment to osmotic pressure, and more of them can increase salt tolerance (Rahneshan et al., 2018). The plants employ a powerful antioxidant defense mechanism to stave off oxidative stress brought on by salinity. Antioxidative enzymes like catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX); organic compounds (including flavonoids, polyphenols, carotenoids, and ascorbic acid); and antioxidant capacity (2,2-diphenyl-1-picrylhydrazyl [DPPH] and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid [$ABTS^{+}$]) are crucial for the detoxification of ROS (Wang et al., 2022). The SOD, which changes superoxide into hydrogen peroxide (H_2O_2), is regarded as a first line of defense. The H_2O_2 is further converted into H_2O by CAT and APX (Islam et al., 2023). Moreover, secondary phytochemicals such as flavonoids, phenols, cyan pigments, tannins, and enzymatic antioxidants boost the total antioxidant capacity of plants by directly neutralizing harmful radicals that are unstable (Azeem et al., 2023; Gengmao et al., 2014). These compounds are also valuable as medicines locally and industrially since they possess a variety of health-improving and disease-prevention qualities.

Nowadays, medicinal plants are significant in sustainable agriculture. Bangladesh is home to medicinal plants that are produced year-round, have lower cultivation expenses, and have less environmental impact. Nevertheless, the shortage of arable land and the fact that most of it is being used to grow food crops to feed her rising population are the results of farmers' unwillingness to cultivate medical plants. Additionally, about 50% of coastal soils eventually become unfit for farming due to their high salt concentration within a year (Dasgupta et al., 2015). The commercial cultivation of several valuable medicinal plants is required to address the growing needs of various ayurveda and pharmaceutical sectors. In this situation, assessing the salt tolerance of potential medicinal plants would be beneficial.

Catharanthus roseus, a member of the Apocynaceae family, represents one of the most popular and commercially important medicinal plants globally, including Bangladesh. All the parts of *C. roseus* can be used for medicinal purposes. It has anti-cancer, anti-diabetic, anti-microbial, anti-fungal, antioxidant, anti-ulcer, anti-helminthic, anti-diabetic, hypotensive, hypolipidemic, and is a great therapy for leukemia and chemotherapy of Hodgkin's disease (S. Das & Sharangi, 2017).

Since this plant has potential uses in pharmaceutical industries in addition to local usage, local farmers are interested in growing and gathering it as a commercial crop. However, how applied stress affects this plant's tolerance systems is unknown. Conversely, it would be challenging for new crops to find room due to the declining quantity of arable land. The present study

assesses to get further insight into how seawater influences biomass production, leaf pigmentation, ion buildup, osmotic adjustment, and antioxidant activity of the *C. roseus* plant. Once this is known, it will be feasible to determine whether *C. roseus* farming may be introduced commercially in the saline-affected areas.

MATERIALS AND METHODS

Plant Growth and the Design of Experiments

A test crop of the important medicinal plant *C. roseus* was used for this research. The experiment was conducted at the Department of Agroforestry and Environment Research Farm, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh, from December 2022 to August 2023. Seeds of *C. roseus* collected from the Bangladesh Rural Advancement Committee (BRAC) nursery were sown first in a seedbed for germination. Afterward, uniformly grown one-month-old seedlings with 4–6 leaves were placed into their corresponding pots on December 10, 2022, which had dimensions of 26.50 cm in height by 27.50 cm in a circle. Previously, the pots were filled with 12.86 kg of a soil mixture with a 4:1 ratio of soil to cow dung manure. A randomized complete block design (RCBD) with five replications was employed in this pot investigation. For salinity treatment, seawater with an electrical conductivity (EC) of 49.60 dS/m was used from Cox's Bazar, a district in southern Bangladesh. The tap water was combined with saltwater to create three different salinity levels (4, 8, and 12 dS/m) that could be reasonably applied to *C. roseus* plants.

Unlike controls, after a one-month adaptation phase, two-month-old *C. roseus* plants were irrigated with 500 cc of diluted seawater every three days for 120 days, whereas the controls received tap water. Salinity treatment was administered progressively at a rate of 4, 8, and 12 dS/m to maintain the appropriate concentration to prevent osmotic shock. The treatment was imposed on February 10, 2023. Each plant received a 4 dS/m saline treatment during the first week, except for the control. Apart from control and 4 dS/m treating plants, all plants were treated with 8 dS/m saline at the end of the second week. Control, 4, and 8 dS/m treated rows of plants received the recommended dosage, whereas other plants were treated with a saline solution containing 12 dS/m after the third week. During the salt stress experiment, the average daytime temperature was 20–34°C, and the average nighttime temperature was 14–26°C. The average humidity was 43–87%.

Measuring Growth Parameters

Data were collected on the plant's height, number of leaves, and number of branches at 60 to 120 days. The total weight of the plant's roots, leaves, and stem was weighed after 120 days as growth markers. The dry weight of a plant's stem and leaves was added to calculate above-ground biomass, also known as shoot dry weight. The roots were thoroughly rinsed with tap water

prior to being measured. Five plants were randomly chosen from each replication of each treatment to evaluate several morpho-physiological and biochemical features at 60, 90, and 120 days after treatment imposition. The first and final data sets were collected on April 10 and June 10, 2023, respectively. All measurements and assays were performed three times for each replicate.

Physiological Parameters

Chlorophyll (Chl) Content and Chlorophyll Stability Index (CSI)

The chlorophylls (Chl *a*, *b*, and total Chls) and the CSI in fresh *C. roseus* leaves were spectrophotometrically determined using the following equation, which was proposed by Sairam et al. (1997) and Witham et al. (1971), respectively:

Chl *a* (mg/g fresh weight)
= [12.70 (D663) - 2.69 (D645)] × [V/1000 × W]

Chl *b* (mg/g fresh weight)
= [22.90 (D645) - 4.68 (D663)] × [V/1000 × W]

Total Chl (mg/g fresh weight)
= [20.20 (D663) - 8.02 (D645)] × [V/100 × W]

CSI = (Total Chl in stressed leaves/Total Chl in control leaves)

where, D (663, 645) = Optical density of the Chl extract at wavelengths of 663 and 645 nm, V = Final volume (ml) of the 80% acetone with Chl extract, and W = Weight of fresh leaf sample in mg.

Estimation of Relative Water Content (RWC)

According to Tamanna et al. (2023), RWC was measured and calculated using the following equation:

Relative water content (RWC) = (FW - DW) / (TW - DW) × 100

where, FW = Fresh weight, DW = Dry weight, and TW = Turgid weight.

Leaf samples were soaked in distilled water for 24 hr to determine the turgid weight (TW), after which they were blotted dry on paper towels and weighed.

Biochemical Parameters

Proline (Pro) and Soluble Sugar Content

According to Bates et al. (1973), the amount of Pro in leaves was determined spectrophotometrically using the acid-ninhydrin method. The Pro content is expressed in µmol/g of fresh weight and was determined by utilizing a standard curve. The amount of total soluble sugars was calculated using the Anthrone method (Yemm & Willis, 1954).

Oxidative Stress Markers

A fresh leaf sample weighing 0.5 g was homogenized in 5 ml of 3% ice-cold trichloroacetic acid (TCA, Merck, Germany) and then the mixture was centrifuged for 20 min at 12,879 × *g* and 4°C. The supernatant was then collected, and its amounts of malondialdehyde (MDA) and H_2O_2 were

estimated. The extract (0.5 ml) was combined with 1 M potassium iodide (1 ml, Merck, Germany) and potassium phosphate buffer (pH 7, 0.5 ml, Merck, Germany) and incubated for 10 min to ascertain the H_2O_2 concentration. The absorbance of the final solution was ascertained at 390 nm according to the Urmi et al. (2023)'s method.

In sealed test tubes containing 0.5 ml of a similar extract, 0.5% 2-thiobarbituric acid (TBA, Sigma-Aldrich, USA) and 0.5 ml of 20% TCA were mixed, and the combination was incubated for 30 min at 95°C in a hot water bath to ascertain the MDA concentration. The samples were submerged in an ice bath, and after the reaction was stopped, they were centrifuged for 10 min at 12,879 × *g*. The absorbances of the resultant supernatant were measured accurately at 532 and 600 nm using a spectrophotometer (Urmi et al., 2023).

A fresh leaf sample (0.5 g) was dissolved in 10 ml of distilled water to assess the electrolyte leakage (EL). The initial readings were obtained using an electric conductivity meter. The samples were sealed and incubated for 30 min in a boiling water bath before the final reading was calculated. The EL percentage was calculated using the Lutts et al. (1996) method.

Extraction and Assessment of Antioxidant Enzymes

A 0.5 g sample of fresh leaf material was broken up in 5 ml (50 mM) of liquid nitrogen and centrifuged at 35,776 × g at 4°C. The retrieved supernatant was used to perform enzyme reactions.

Catalase Activity (CAT)

The H_2O_2 (15 mM, Sigma-Aldrich, USA) and potassium phosphate buffer (50 mM, Merck, Germany) were combined with enzyme extract (50 L). The mixture's initial absorbance was immediately noted, and the subsequent decline in absorbance was noted after 1 min. The CAT activity was computed utilizing the Noctor et al. (2016)'s method.

Ascorbate Peroxidase Activity (APX)

A reaction mixture comprising 0.1 mM H_2O_2, 0.55 mM ascorbic acid (Sigma-Aldrich, USA), and a phosphate buffer solution (50 mM, pH 7, Merck, Germany) was generated in the absence of light (Nakano & Asada, 1981). Results were recorded as soon as the reaction mixture and enzyme extract (50 L) were mixed.

Total Antioxidant Capacity

Radical Scavenging Activity of DPPH

Assay for evaluating total antioxidant activity using DPPH radical scavenging (Abdul-Hafeez et al., 2014). One gram of plant samples were homogenized with 5–10 ml of HPLC-grade methanol (Merck, Germany) using a mortar and pestle to a uniform consistency. The samples were centrifuged at 805 × g for 20 min at 40°C, with the supernatants being kept at -20°C for further investigation. After that, 1 ml of the methanolic sample extract was obtained, and 1 ml of the DPPH solution (Sigma-Aldrich, USA) with the equivalent solvent devoid of plant material was taken in another test tube to serve as the control. Each test tube received 3 ml of a 0.2 mM DPPH solution,

and the reaction mixture was then incubated at 20°C for 5 min. The mixture's absorbance was then compared to a blank (methanol) at a wavelength of 517 nm. The amount of DPPH radicals each sample could scavenge was determined using the equation below.

$$\text{Inhibition (I) \%} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \times 100\%$$

The antioxidant activity was calculated using a standard curve, and the antioxidant capacity (µg/g FW) was reported as the equivalent amount of ascorbic acid.

Radical Scavenging Activity of $ABTS^+$

The $ABTS^+$ radical scavenging analysis was performed using a modified Cai et al. (2004) methodology. In a nutshell, 2.45 mM potassium persulfate solution (Sigma-Aldrich, USA) and 7 mM $ABTS^+$ stock solution (Merck, Germany) were reacted at a ratio of 0.5:1 to produce $ABTS^+$ radical cations. They were then permitted to stand for 12 to 16 hr at room temperature in the dark. The absorbance of the $ABTS^+$ solution was adjusted by adding 80% ethanol (Merck, Germany) until a reading of 0.700 ± 0.05 was obtained at 734 nm. 0.1 ml of diluted leaf extract was used in a reaction that used 3.9 ml of blue green $ABTS^+$ solution. After 6 min, the solution's absorbance at 734 nm was determined. Trolox standard solution (Merck, Germany) was employed to create standard calibration. The test results, which were run in triplicate, were expressed as Trolox equivalent antioxidant capacity (TEAC, µmol of Trolox equivalent antioxidant capacity based on dry weight, or µmol TEAC/g DW).

$$\text{Inhibition (I) \%} = \text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}} \times 100\%$$

Total Phenol and Total Flavonoid Content

To calculate the total phenolic content (TPC), 0.3 ml of plant extract was combined with 0.15 ml of the 10% Folin-Ciocalteu reagent (Sigma-Aldrich, USA). After 5 min, the reaction mixture was added to a saturated sodium carbonate solution (7.5%) and then incubated for 90 min. After measuring the absorbance at 765 nm, the phenolic content was determined using gallic acid as a reference.

To quantify the total flavonoid concentration (TFC), methanolic extracts (250 µl) were mixed with 10% aluminum chloride (50 µl, Sigma-Aldrich, USA), potassium acetate (50 µl, Sigma-Aldrich, USA), and distilled water (1.4 ml) before being incubated for 40 min. The absorbance was determined at 510 nm using quercetin as a standard.

Abdul-Hafeez et al. (2014) and Zhishen et al. (1999) provided the methodologies used to determine the amounts of total phenol and flavonoid, respectively.

Ion Content Measurement in Leaves and Roots

An atomic absorption spectrophotometer (Hitachi, Model: 170-30, Japan) was used to estimate the concentrations of Na^+, K^+, Ca^{2+}, and Mg^{2+} ions in the oven-dried samples

of roots and shoots using the techniques described by Mostofa et al. (2015).

Data Analysis

The data was examined using one-way analysis of variance (ANOVA) using Statistix 10 software. To ascertain whether treatments had statistically significant differences ($p < 0.05$), the least significant difference (LSD) test was performed using Statistix 10 software. Different letters were used to represent these changes. Five biological replications (n = 5) were used to acquire each treatment's values (means ± SEs); the findings are displayed in the tables and figures. Microsoft Excel (version 16.78.3, Vol. License 2019) was used for data calculation, analysis, and subsequent graphical presentation. Minitab was used for the dendrogram, and Origin Pro 9 was used for principal component analysis (PCA).

RESULTS

Alternations of the Growth and Biomass of *C. roseus* under Seawater Stress

Catharanthus roseus plants exposed to seawater exhibited significant phenotypic interruption, including stunted growth, decolorization of leaves (which turned pale and yellow), and reduction in total fresh weight (TFW) and total dry matter (TDM) upon increasing the degree of seawater for all the sampling dates (Table 1; Figure 1). On day 120, the tallest (108.60 cm) and shortest plants (68.20 cm) were measured in the control and 12 dS/m saline treatments, respectively. Plants exposed to seawater at doses of 4, 8, and 12 dS/m experienced substantial height reductions of 5.5, 9.9, and 19.2% at day 60; 6.8, 14.4, and 34.2% at day 90; and 8.6, 18.0, and 46.4% at day 120, respectively, as compared to the comparable control values (Table 1). Seawater-induced salinity reduced the number of leaves per plant by 5.4, 11.6, and 29.4% at day 90 and 7.4, 16.7, and 36.4% at day 120 when planted under 4, 8, or 12 dS/m salinity levels, respectively. Salt stress brought on by the seawater also significantly negatively affected the number of branches per plant.

The percentage of branch reduction was the lowest (16.3%) at 4 dS/m while the highest (49%) in maximum salinity levels (12 dS/m) at 120 days after treatment application, compared to the unstressed control plants (Table 1). It is apparent from Table 2 that salinity significantly reduced the shoot and root biomass of *C. roseus*. At day 120, seawater with high salinity (12 dS/m) lowered shoot FW and DW by 53.4 and 61%, while root FW and DW decreased by 71.74 and 75.35%, respectively, compared to the corresponding controls (Table 2). Irrigation with seawater having 4 and 12 dS/m salinity reduced TFW by 12.66 and 54.5%, respectively, over their respective controls at day 120 (Table 2). The least and maximum percent reduction of TDM was recorded for the parameter at 4 dS/m (14.65%) and 12 dS/m (72.34%) salinity levels, respectively, as opposed to the control. There is a minimal reduction in TFW and TDM with moderate salinity, 23 and 25%, respectively. Also, with rising seawater treatment, the root/shoot ratio for fresh and dry biomass rose (Table 2).

Figure 1. Phenotypic appearance of *Catharanthus roseus* plants: control, seawater treatment (4, 8, and 12 dS/m)

Table 1
Impact of different salt levels generated by seawater on the morphological attributes of Catharanthus roseus *at different times following treatment imposition*

Days after treatment initiation	Salinity levels (dS/m)	Plant height (cm)	No. of leaves	No. of branches
60	Control	86.20 ± 1.16c	210.23 ± 2.53a	37.00 ± 0.37a
	4	81.40 ± 0.75c	201.21 ± 2.24b	30.00 ± 0.55b
	8	77.60 ± 0.93d	194.83 ± 2.47c	26.00 ± 0.55c
	12	69.20 ± 0.66e	183.42 ± 2.76d	23.00 ± 0.60d
CV (%)		2.03	2.70	3.24
90	Control	99.60 ± 1.03a	251.22 ± 1.65a	44.00 ± 0.37a
	4	92.80 ± 0.86b	237.61 ± 1.72b	39.00 ± 0.37b
	8	85.20 ± 1.32d	221.84 ± 2.47c	31.00 ± 0.68c
	12	61.60 ± 0.68e	177.43 ± 2.13d	25.00 ± 0.37d
CV (%)		2.83	2.05	3.18

Table 1 *(Continue)*

Days after treatment initiation	Salinity levels (dS/m)	Plant height (cm)	No. of leaves	No. of branches
120	Control	108.60 ± 1.08a	298.55 ± 1.9a	55.00 ± 0.40a
	4	99.20 ± 0.86b	276.32 ± 2.4b	46.00 ± 0.58b
	8	89.00 ± 1.58c	248.62 ± 1.6c	40.00 ± 0.66c
	12	58.20 ± 0.58f	189.61 ± 2.2d	28.00 ± 0.45d
CV (%)		3.99	2.32	3.20

Note. Data represents mean ± standard error of 5 independent replicates. The significant differences at $p < 0.05$ are indicated by different letters

Table 2
Impact of different salt levels generated by seawater on the shoot and root biomass of Catharanthus roseus *at 120 days following treatment imposition*

Salinity level (dS/m)	Shoot fresh weight (g)		Root/ Shoot	Root fresh weight (g)	TFW (g)	Shoot dry weight (g)		Root dry weight (g)	Root/ Shoot	TDM (g)
	Leaf fresh weight (g)	Stem fresh weight (g)				Leaf dry weight (g)	Stem dry weight (g)			
Control	90.54 ± 0.46a	375.40 ± 2.13a	0.15 ± 0.32c	70.90 ± 0.84a	530.84 ± 1.60a	37.78 ± 0.44a	130.94 ± 0.86a	31.00 ± 0.84a	0.18 ± 0.12c	204.72 ± 0.50a
4	82.24 ± 0.63b	317.11 ± 0.87b	0.16 ± 0.24	64.26 ± 0.76b	463.61 ± 1.50b	33.22 ± 0.46b	115.70 ± 0.66b	28.80 ± 0.76b	0.19 ± 0.22b	174.72 ± 1.10b
8	73.78 ± 0.51c	285.65 ± 1.48c	0.17c ± 0.56b	59.76 ± 0.61c	409.19 ± 2.44c	24.42 ± 0.37c	103.62 ± 0.44c	24.58 ± 0.61c	0.19 ± 0.15b	152.62 ± 0.16c
12	59.62 ± 0.51d	151.71 ± 0.89d	0.22 ± 0.21a	45.97 ± 1.17d	241.30 ± 1.57d	10.42 ± 0.57d	46.81 ± 0.37d	11.38 ±1.17d	0.20 ± 0.23a	56.61 ± 0.84d
CV (%)	3.64	4.46	3.33	3.45	1.15	3.36	1.71	2.46	2.19	4.31

Note. Data represents mean ± standard error of 5 independent replicates. The significant differences at $p < 0.05$ are indicated by different letters. TFW = Total fresh weight; TDM = Total dry matter

Alteration in the Physiological Responses of *C. roseus* to Seawater Stress

The amounts of Chl *a*, Chl *b*, and total Chl in the leaves of *C. roseus* plants treated with various salinities induced by saltwater decreased progressively and noticeably (Table 3). The Chl *b* content of leaves rose with the passage of time up to 90 days under salinity treatments of 4 and 8 dS/m and, after that, progressively declined. After applying low (4 dS/m), moderate (8 dS/m), and high salinity (12 dS/m), the Chl *a* content was reduced by 12, 25, and 43%, the Chl *b* content by 6.7, 11.5, and 32%, and the total Chl content by 10.5, 22, and 41% at day 120. Under severe salt stress (12 dS/m) at day 120, the reduction of Chl *a* (by 43%) was larger than the reduction of Chl *b* (by 32%), resulting in a noticeably lower level of overall Chl content (41%).

As evident from Table 3, seawater stress had a negative effect on the Chl stability index (CSI) of the *C. roseus* plant. The CSI dropped 33.66%, whereas the salt level went up from 4 to 12 dS/m at day 120. Under stress from seawater, the RWC of *C. roseus* leaves was comparatively higher early in the growth cycle than at a later stage. RWC was found to be decreased by 5.31, 16.43, and 25.76% at day 90 and 7.7, and 22.90 and 35.24% at day 120 under 4, 8, and 12 dS/m salinity levels, respectively, in contrast to the relative control value.

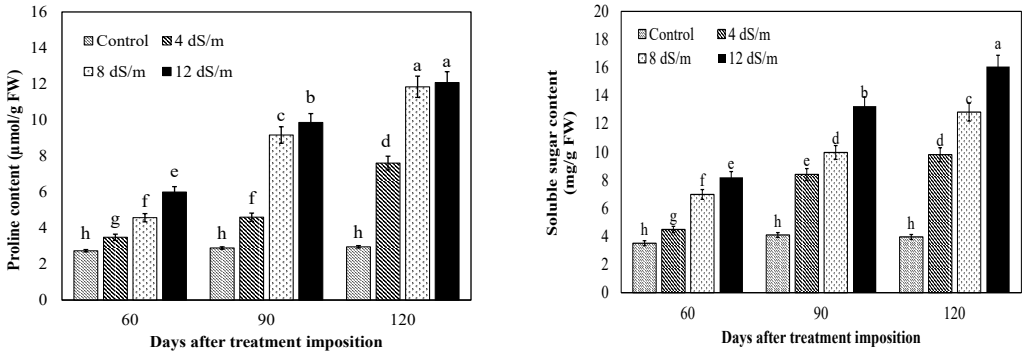
Table 3
Impact of different salt levels generated by seawater on chlorophyll a *(Chl* a*), chlorophyll* b *(Chl* b*), total chlorophyll (Chls), chlorophyll stability index (CSI), and relative water content (RWC) of* Catharanthus roseus *leaves at different times following treatment imposition*

Days after treatment imposition	Salinity levels (dS/m)	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Total Chl (mg/g FW)	CSI (%)	RWC (%)
60	Control	3.22 ± 0.02a	0.95 ± 0.06a	4.17 ± 0.14a	-	90.96 ± 0.34a
	4	2.95 ± 0.01b	0.98 ± 0.04b	3.93 ± 0.21b	94.24 ± 0.33b	87.44 ± 0.39b
	8	2.90 ± 0.02c	1.04 ± 0.02c	3.94 ± 0.15c	94.48 ± 0.25c	79.95 ± 0.42c
	12	2.68 ± 0.01d	0.81 ± 0.01d	3.49 ± 0.23d	83.69 ± 0.74d	72.74 ± 0.23d
90	Control	3.38 ± 0.02a	0.98 ± 0.01a	4.36 ± 0.12a	-	91.78 ± 0.23a
	4	3.11 ± 0.01b	1.08 ± 0.03b	4.19 ± 0.18b	96.10 ± 0.23b	86.90 ± 0.33b
	8	2.75 ± 0.04c	1.12 ± 0.01b	3.87 ± 0.25c	88.76 ± 1.37c	76.70 ± 0.19c
	12	2.31 ± 0.01d	0.79 ± 0.03c	3.10 ± 0.14d	71.10 ± 0.67d	68.14 ± 0.27d
120	Control	3.54 ± 0.01a	1.04 ± 0.01a	4.58 ± 0.11a	-	92.65 ± 0.22a
	4	3.13 ± 0.03b	0.97 ± 0.03b	4.10 ± 0.32b	89.51 ± 0.20 b	85.51 ± 0.21b
	8	2.66 ± 0.01c	0.92 ± 0.03c	3.58 ± 0.16c	78.16 ± 1.45c	71.46 ± 0.26d
	12	2.01 ± 0.01d	0.71 ± 0.10 d	2.72 ± 0.12d	59.38 ± 0.60d	60.00 ± 0.27e
CV (%)		5.10	3.81	2.16	3.41	3.77

Note. Data represents mean ± standard error of 5 independent replicates. The significant differences at $p < 0.05$ are indicated by different letters

Alternations in the Biochemical Responses of *C. roseus* to Seawater Stress Soluble Sugar and Pro Content

To comprehend the mechanisms behind osmotic adjustment, the quantities of Pro and soluble sugars in *C. roseus* plants at various salinity levels were examined. The seawater stress caused a considerable rise in Pro and soluble sugar levels at all sampling dates (Figure 2). *C. roseus* rapidly increased leaf Pro as salinity increased from low to moderate and high salinities (8 and 12 dS/m) (216 and 241%) at day 90 and statistically remained constant (301 and 309%) at day 120, respectively, as compared to control. As opposed to controls, soluble sugars steadily increased at days 60, 90, and 120 under moderate (8 dS/m) (98, 143, 225%) and high (12 dS/m) (133, 224, 306%) salinity conditions, respectively (Figure 2).



(a) (b)

Figure 2. Impact of different salt levels generated by seawater on the (a) proline (Pro) and (b) soluble sugar content of *Catharanthus roseus* at different times following treatment imposition

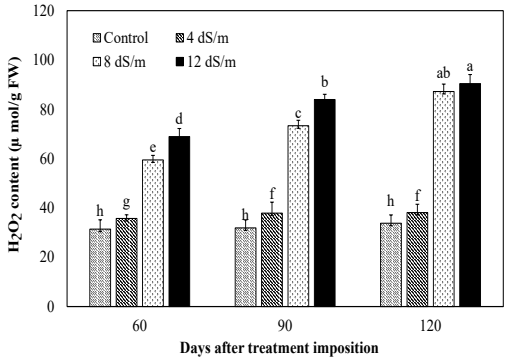
Note. Means and standard errors are displayed using bars (n = 5). The significant differences at $p < 0.05$ are indicated by different letters

Generation of Oxidative Stress Indicators

Oxidative stress indicators such as H_2O_2, MDA, and EL increased with salinity, reaching the highest levels at 12 dS/m salinity at day 120 (Figure 3). The H_2O_2 concentration increased significantly at moderate and severe salinities (8 and 12 dS/m) by 89, 119% at day 60; 129, 163% at day 90; and 158, 167% at day 120, respectively, compared to the relative control value, whereas at low salinities (4 dS/m), there were no discernible changes in H_2O_2 contents (Figure 3a). Mild (4 dS/m) and moderate salinity (8 dS/m) had little effect on MDA levels at days 60 and 90. However, after being exposed to saltwater at concentrations of 4, 8, and 12 dS/m for 120 days, there was an apparent rise in MDA content (175, 204, and 258%) compared to the control (Figure 3b). The EL concentration was not pointedly impacted by low or moderate salinity at any of the sampling dates; however, the percentage increase was only found to be highest at high salinity (by 194, 251, and 622% at days 60, 90, and 120, respectively) compared to control (Figure 3c).

Alternations of Antioxidant Compounds, Capacity, and Enzymes under Seawater Stress

The antioxidant defense mechanism, which is composed of enzymes that are antioxidant (CAT and APX), antioxidant ability (DPPH and $ABTS^+$), and antioxidant substances (total phenols and flavonoids), has significantly increased upon increasing seawater stress (Table 4; Figure 3). The activity of CAT and APX progressively rose as salt concentrations and exposure periods were increased (Figures 3d and 3e). On days 60 and 90, CAT increased at moderate (40 and 70%) and high (69, 139%) salinities (Figure 3d), whereas APX (99 and 138%) significantly increased only at high salinities, respectively, in comparison to the control (Figure 3e). After



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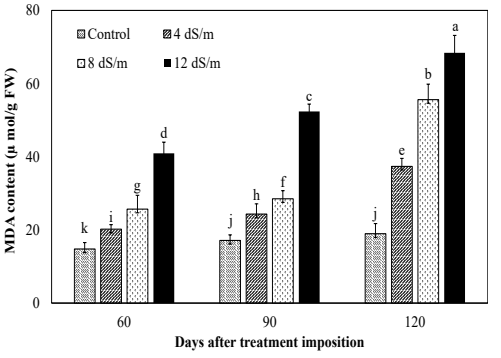

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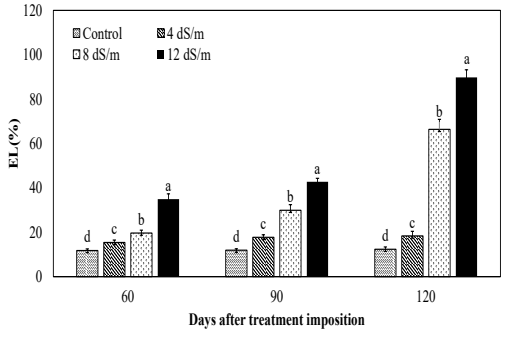

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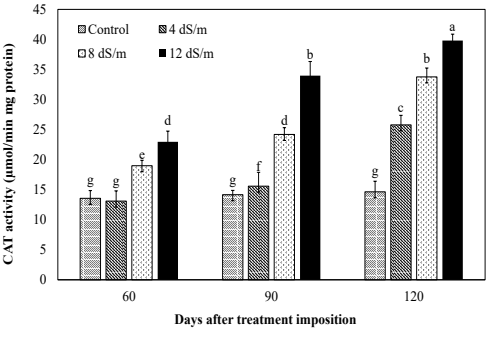

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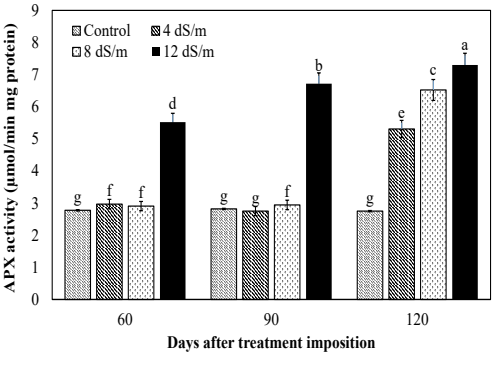


(e)

Figure 3. Impact of different salt levels generated by seawater on (a) hydrogen peroxide (H_2O_2), (b) malondialdehyde (MDA), (c) electrolyte leakage (EL) content, (d) catalase (CAT), and (e) ascorbate peroxidase (APX) activity of *Catharanthus roseus* at different times following treatment imposition

Note. Means and standard errors are displayed using bars (n = 5). The significant differences at $p < 0.05$ are indicated by different letters

the seawater exposure time was prolonged for an additional 30 days, CAT activity was elevated by 76, 130, and 171% and APX activity by 93, 137, and 165% at salinity indices of 4, 8, and 12 dS/m, respectively, as opposed to control. There was no substantial variation in DPPH and $ABTS^+$ activities at low salinity; however, at both moderate and high salinity (8 and 12 dS/m), the activities of DPPH (138 and 201%) and $ABTS^+$ (78 and 123%) considerably increased on day 90 as compared with control. Alternatively, DPPH activities were further increased by 198, 234%, and $ABTS^+$ 100, 192%, at intermediate (8 dS/m) and severe (12 dS/m) salinity levels on day 120, respectively, in contrast to control. The increasing rate of phenol content was found to be 102.28,

280.7, and 275.4% of control under 4, 8, and 12 dS/m salinities, respectively, indicating that the rate of accumulation of TPC was higher at the 8 dS/m treatment level at day 120. The increment of TFC displayed the greatest at the severe salinity level (12 dS/m); the TFC was 100.54 and 105.94% higher at days 90 and 120, respectively, as opposed to salt-free control plants.

Table 4
Impact of different salt levels generated by seawater on total antioxidant activity, TFC, and TPC of Catharanthus roseus *leaves at different times following treatment imposition*

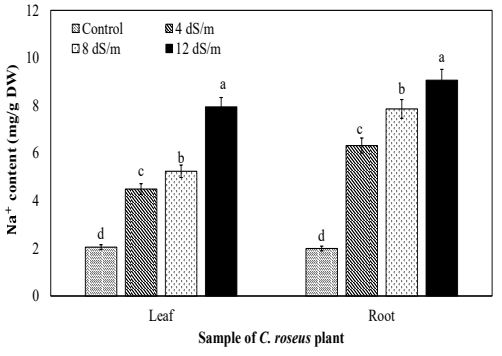
Days after treatment imposition	Salinity levels (dS/m)	Total antioxidant activity (DPPH) (µg/g FW)	Total antioxidant activity ($ABTS^+$) (µg/g FW)	Total flavonoid content (TFC) (mg of quercetin/g FW)	Total phenol content (TPC) (mg/g GAE FW)
60	Control	22.95 ± 0.40g	40.22 ± 1.94f	1,242.57 ± 7.02ef	228.97 ± 5.45h
	4	23.09 ± 0.75g	44.84 ± 1.66f	1,362.43 ± 14.57e	321.43 ± 3.92g
	8	38.74 ± 0.62e	61.67 ± 1.62e	1,791.83 ± 9.50d	383.43 ± 8.98f
	12	46.76 ± 0.49d	80.33 ± 2.91c	2,023.97 ± 13.38c	552.97 ± 9.85d
90	Control	22.36 ± 0.41g	42.19 ± 1.64f	1,304.71 ± 6.59e	210.71 ± 4.40h
	4	26.29 ± 0.29f	45.28 ± 3.74f	1,558.19 ± 11.45d	331.19 ± 2.17g
	8	53.38 ± 0.48c	75.32 ± 2.84d	1,904.76 ± 6.83c	440.76 ± 8.76e
	12	67.36 ± 0.41b	94.18 ± 2.44b	2,616.59 ± 11.22b	689.59 ± 8.20c
120	Control	23.20 ± 0.37g	44.21 ± 2.01f	1,467.40 ± 7.28e	207.00 ± 5.09h
	4	27.98 ± 0.33f	68.76 ± 1.15d	1,602.73 ± 5.45d	418.73 ± 6.03ef
	8	69.36 ± 0.74b	88.52 ± 1.53c	2,125.82 ± 15.50c	788.11 ± 4.73a
	12	77.60 ± 0.57a	129.43 ± 5.81a	3,022.11 ± 13.69a	777.22 ± 8.62b
CV (%)		4.56	3.91	5.97	5.33

Note. Data represents mean ± standard error of 5 independent replicates. The significant differences at $p < 0.05$ are indicated by different letters

Mineral Content

The study examined the levels of Na^+, K^+, Ca^{2+}, and Mg^{2+} in the roots and leaves to comprehend *C. roseus*'s ion homeostasis strategy on day 120 of the varied seawater treatments. It was clear that saline stress had a remarkable effect on Na^+ content in leaves and roots and that this effect grew with increasing salinity. However, the content of Na^+ was found to be lower in leaves than in roots. Figure 4a demonstrates that treatment of *C. roseus* plants with various seawater levels (4, 8, and 12 dS/m) significantly increased the content of Na^+ (68, 74, and 78% in roots and 54, 61, and 74% in leaves, respectively). Leaf K^+ concentration increased (7.5%) in 4 dS/m salinity treatments, despite being statistically similar to the control, before starting to drop (Figure 4b). It is pertinent to note that leaves have been shown to have lower Na^+ concentrations and higher K^+ concentrations than roots, indicating that *C. roseus* has a greater ability to withstand salt. It also noticed a considerable drop in the amounts of Ca^{2+} and Mg^{2+} in the

leaves and roots of seawater-stressed plants compared to control plants grown without access to seawater (Figures 4c and 4d). In addition, plants stressed by seawater had substantially larger amounts of Ca^{2+} and Mg^{2+} in their leaves than in their roots. The K^+/Na^+ ratios were assessed and found that when salt levels steadily increased, the ratios in both leaves and roots tended to trend lower. Notably, leaves, as opposed to roots, showed greater K^+/Na^+ ratios (Figure 4e). *Catharanthus roseus* maintained larger amounts of K^+, Ca^{2+}, and Mg^{2+} in leaves and modified its ion homeostasis during salt stress because of the retention of Na^+ in roots.



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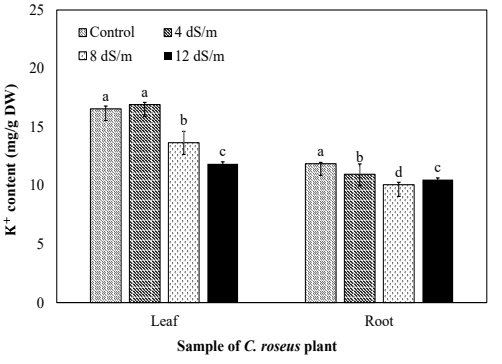

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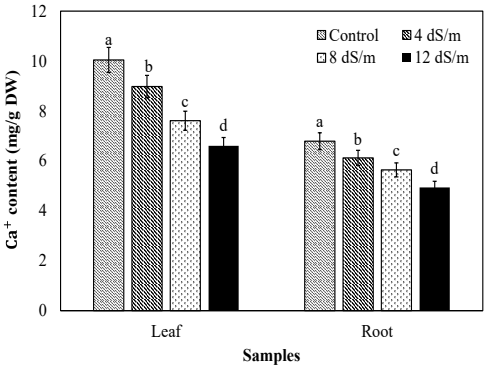

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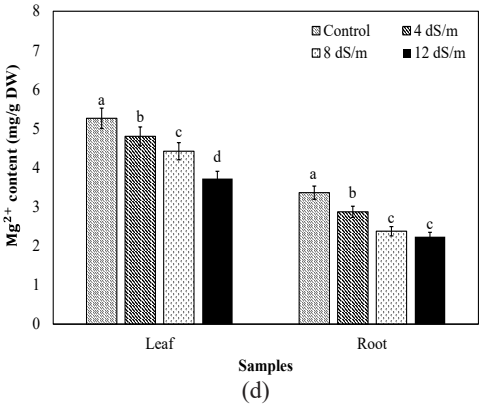

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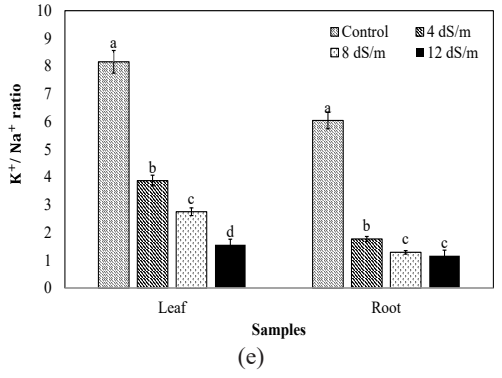


(e)

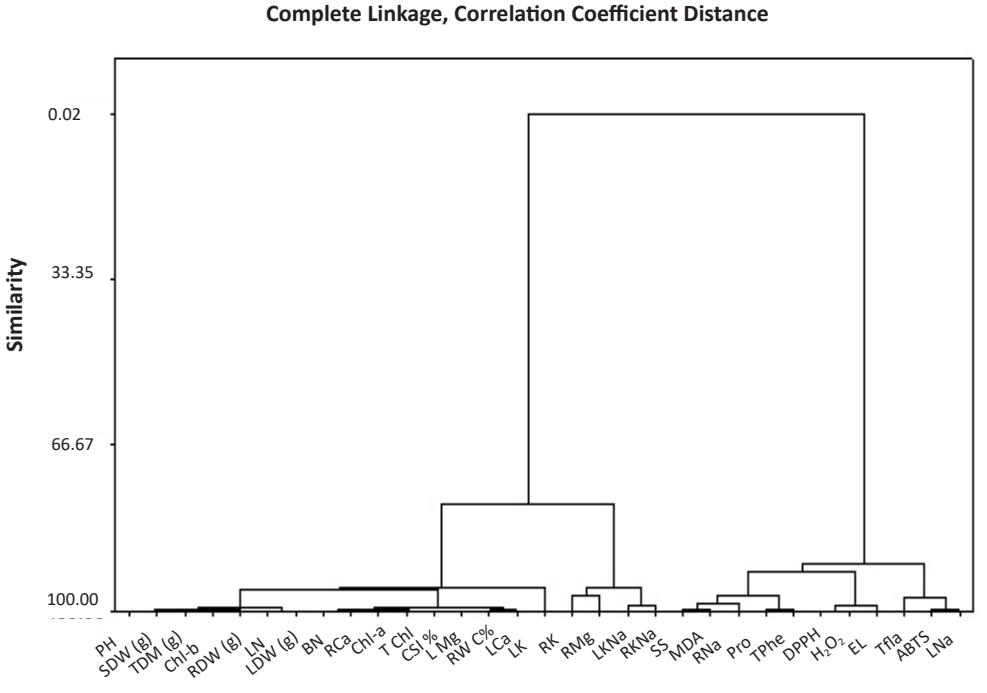
Figure 4. Impact of different salt levels generated by seawater on (a) Na^+, (b) K^+, (c) Ca^{2+}, (d) Mg^{2+}, and (e) K^+/Na^+ ratio of *Catharanthus roseus* plants at 120 days following treatment imposition

Note. Means and standard errors are displayed using bars (n = 5). The significant differences at $p < 0.05$ are indicated by different letters

Data from Morpho-physiological and Biochemical Processes are Displayed Using a Dendrogram and PCA

To evaluate the gathered data rapidly, a clustered dendrogram was generated. Four clusters are generated in the dendrograms A (total flavonoid [Tfla], ABTS^{+}, leaf Na^{+} [LNa^{+}]), B (soluble sugar [SS], MDA, root Na^{+} [RNa^{+}], Pro, total phenol [TPhe], DPPH, H_2O_2), C (root K^{+} [RK^{+}], root Mg^{2+} [RMg^{2+}], leaf K^{+}/Na^{+} [LK^{+}/Na^{+}], root K^{+}/Na^{+} [RK^{+}/Na^{+}] ratio), and D (plant height [PH], shoot dry weight [SDW], total dry matter [TDM], Chl *b*, root dry weight [RDW], leaf number [LN], leaf dry weight [LDW], branch number [BN], root Ca^{2+} [RCa^{2+}], Chl *a*, total Chl [TChl], CSI, leaf Mg^{2+} [LMg^{2+}], leaf Ca^{2+} [LCa^{2+}], leaf K^{+} [LK^{+}]). Cluster A was strongly linked with cluster B, showing a similarity of about 90. On the other hand, the C and D clusters are linked, showing a similarity of about 80. Among the four clusters, variables are very strong in the D cluster, followed by B, A, and C. Variables from clusters A and B revealed an increased trend when plants were subjected to 8 and 12 dS/m of salt stress compared to the control treatment employing seawater-free circumstances. As opposed to untreated control plants, cluster C and D variables obtained at 4, 8, and 12 dS/m of salt stress showed declining trends (Figure 5a).

PCA was used to analyze the correlations between the treatments and the different variables (Figure 5b). When combined, the PCA1 and PCA2 scores explained 97.64%



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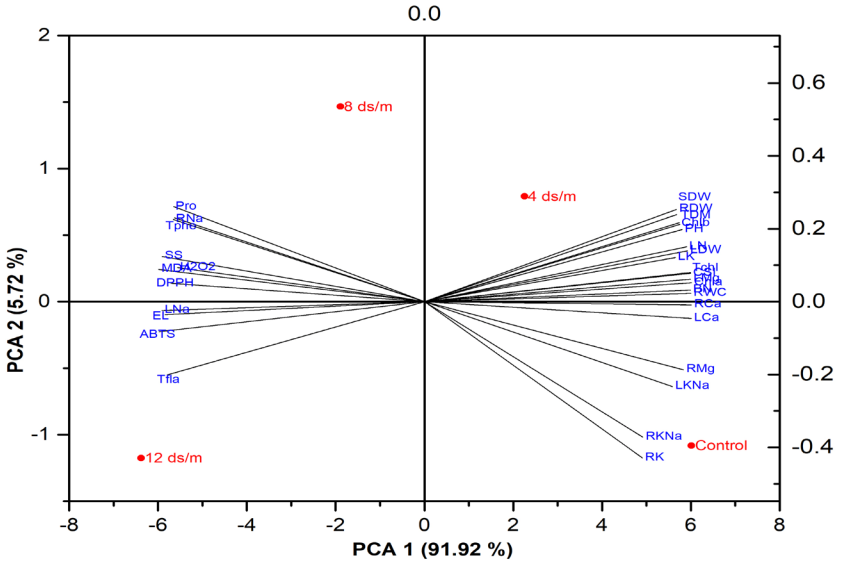

(b)

Figure 5. (a) Dendrogram and cluster analysis of growth, physiological, and biochemical traits of *Catharanthus roseus* plant; (b) principal component analysis (PCA) was employed to make links between treatments and variable levels clearer

Note. The variables included PH (plant height), LN (leaf number), BN (branch number), TFW (total fresh weight), TDM (total dry matter), RWC (relative water content), LCa^{2+} (leaf calcium content), RCa^{2+} (root calcium content), Chl *a* (chlorophyll *a*), Chl *b* (chlorophyll *b*), TChl (total chlorophyll), LMg^{2+} (leaf magnesium content), RMg^{2+} (root magnesium content), LK^{+} (leaf potassium content), RK^{+} (root potassium content), SS (soluble sugar), LK/Na (leaf K/Na ratio), RK/Na (root K^{+}/Na^{+} ratio), CSI (chlorophyll stability index), MDA (malondialdehyde), LNa^{+} (leaf sodium content), RNa^{+} (root sodium content), Pro (proline), TPhe (total phenol), Tfla (total flavonoid), and H_2O_2 (hydrogen peroxide), respectively

of the variability in the data. A PCA biplot showed that PCA1 contributed positively to physiological traits like H_2O_2, Pro, TPhe, DPPH, SS, and RNa^{+}, accounting for around 91.92% of the total variability, and negatively to factors like LNa^{+}, EL, $ABTS^{+}$, and Tfla, accounting for 5.72% of the total variation.

DISCUSSION

Effect of Seawater-induced Salinity on the Growth Parameters

Plants expend enough energy on various metabolic functions to adapt to environmental challenges. Salt stress has a major impact on plant metabolism and development. According to the present investigation, seawater reduced the growth performance of *C. roseus* by interfering with various traits essential to its growth. These metrics were plant height, the quantity of leaves, branches and shoots, and root FW and DW, which were strongly affected by increasing the levels of salinity (Tables 1 and 2; Figure 1). A higher accumulation of sodium chloride in the cytosol and cell membrane of *C. roseus* leaves may result in fewer leaves. Concurrently, the cell sap

on the leaves might not have been able to retain more salt at the same time, which led to a decrease in the salt content of the cells and a rapid withering of the leaves (Haddadi et al., 2016).

In agreement with our findings, Obaidullah et al. (2022) and S. Das et al. (2024) reported that elevated salinity levels reduced the number of leaves in *Vitex negundo* and *Justicia adhatoda*. Likewise, it was also evident that rising salinity had a detrimental effect on the emergence of new buds. The decline in branches was brought on by steadily increasing salinity, which is corroborated by studies on *J. adhatoda* by Obaidulahah et al. (2022), *Zea mays* by Hassanein et al. (2009), and *Withania somnifera* by Jaleel et al. (2008). According to Table 2, the present investigation illustrates a negative correlation between seawater stress and TFW and TDM, which is in line with the findings of Azeem et al. (2023) and Li et al. (2022). A general drop in DW has been shown in all *C. roseus* plant tissues under salt stress; however, this decrease is particularly noticeable in the aerial section of the plant. This drop in shoot biomass of seawater-induced *C. roseus* plants could be attributed to insufficient nutrient availability in the growth medium, a slower rate of water entry into the plants, a decline in photosynthetic output, and a suppression of CO_2 supply.

It can also be explained that the considerable drop in TFW and TDM of *C. roseus* can be attributed to reduced photosynthesis, a stagnant or reduced mobilization of reserve foods, and an interruption of plant cell division (Alam et al., 2015). Extreme salinity significantly impairs cell proliferation and growth, meristematic function, carbon sequestration, the distribution of photoassimilation among roots, stems, and leaves, as well as both nutrients and water absorption (Pan et al., 2016; Rahman et al., 2017), all of which lead to decreased growth performance, as was observed in this experiment. In accordance with previous reports, certain species can withstand moderate salinity, exhibiting growth reductions ranging from 25 to 50% (Cassaniti et al., 2012). *C. roseus* can withstand 8 dS/m salt successfully by retaining its biomass accumulation in both the root and the shoot, only experiencing a small decrease in the overall fresh (23%) and dry (25%) biomass (Table 2) compared to the respective control. The finding is also supported by similar research on *V. negundo* and moringa (Bekka et al., 2022; S. Das et al., 2024).

However, TFW (54%) and TDM (72%) of *C. roseus* dramatically decreased at severe salinity (12 dS/m). Excessive salt content in the rooting media causes a physiological drought by lowering the water content of the tissue and limiting the amount of water available before it becomes ion-toxic. A plant may utilize half of its energy to regulate metabolic processes and the remaining to grow and produce biomass (Uematsu et al., 2012). The study suggests that moderate salinity (8 dS/m) may reduce the overall amount of energy generated by photosynthesis and redirect a significant portion of that energy into stress-regulatory processes rather than development.

Physiological Traits Caused by Seawater Salinity and Their Impact

It was noticed that *C. roseus* plants exhibit a decrease in photosynthetic pigments, primarily Chl *a* and Chl *b*, essential to photosynthesis, with an increment in seawater concentration and exposure time (Table 3). Chl degradation was observed more than synthesis under salinity stress. It can be explained by the development of proteolytic enzymes like chlorophyllase, which degrade Chl while also harming the photosynthetic apparatus (Isayenkov & Maathuis, 2019), reduce the plant photosynthesis process (Mafakheri et al., 2010), and prevent the buildup of accumulated ions (Jaleel et al., 2008). The study results are in accordance with findings from research on pepper and common beans by Abdelhamid et al. (2013) and Hand et al. (2017), respectively.

The decrease in total Chl concentration, especially at moderate salinity (8 dS/m) levels, suggests a way to prevent excessive ROS generation and shield the photosynthetic machinery from salt-induced photodamage (Farooq et al., 2022). *C. roseus* also had a similar pattern of pigment loss when exposed to salt (Table 3). According to Acosta-Motos et al. (2017), salt tolerance of the plant affects the Chl content, even though it decreases during salt stress. The disruption of the photosynthetic mechanism, the instability of pigment-protein complexes, the dysfunction of pigments, and structural damage to the light-harvesting complex may all be contributing factors to the decline in total Chls (Geissler et al., 2009). Additionally, excessive salt concentrations cause chloroplasts to generate ROS that breaks the multiple bonds of fatty acids that are unsaturated, damages the membrane of the chloroplast, and lets Chl spill out of the thylakoids (Sun et al., 2010). Our results also revealed that Chl *a* is more sensitive to salinity than Chl *b*, in accordance with past investigations of Gururani et al. (2015). They reported that the most abundant and necessary part of the light-harvesting complex is Chl *a*, while Chl *b* serves as a supplemental pigment and incidentally aids in photosynthesis by transmitting photons to Chl *a*. The prevailing consensus is that the faster breakdown of their pigment, impeded synthesis, and rapid plastid degradation are the causes of these pigments' decline under salt stress (Chen, 2014). Thus, *C. roseus* may have survived the salt-induced loss of photosynthetic activity by having more Chl *a* than Chl *b* at low and moderate seawater concentrations (Table 3), as shown in *V. negundo* by S. Das et al. (2024). Nevertheless, at high salinity (12 dS/m), *C. roseus* leaves lost 41% of their total Chl content, suggesting a major threat to the plant's survival.

With time and rising salinity, salt stress reduced the RWC of *C. roseus* compared to the control (Table 3). This decline could be the result of salt-induced restrictions on water accessibility and intake as well as damage to the root system of *C. roseus*. By building salt in the root zones and limiting water intake, salt stress lowers the root water potential and may result in osmotic imbalances (Kumar et al., 2021). According

to Bistgani et al. (2019), a drastic change in plant water status under saline conditions may be due to high concentrations of Na^+ uptake in plants and to delay the osmoregulation of the salinity tolerance threshold. These findings are consistent with other studies conducted on the plants *Prosopis alba* (Meloni et al., 2004), *Z. mays* (Çiçek & Çakirlar, 2002), and *Shepherdia argentea* (Qin et al., 2010).

Biochemical Traits Caused by Seawater Salinity and Their Impact

Pro and Soluble Sugar Content

In cellular metabolism, salt toxicity was avoided by organic osmolyte accumulation, which greatly assisted with osmotic adjustments, preserved turgidity, and prevented cellular metabolism from salt toxicity (S. Das et al., 2024). *C. roseus* accumulates greater Pro (Figure 2a), which may significantly play a role in osmotic adaptation and tolerance to salt as observed in different plant species (Azeem et al., 2019; S. Das et al., 2024; Sharif et al., 2018). The increased Pro could regulate the cytosolic pH buffer for subcellular structures, scavenge singlet oxygen and hydroxyl radicals, and lessen the acidification of cells caused by salinity stress (Isayenkov & Maathuis, 2019). Furthermore, the fact that elevated Pro concentrations build up in the cytosol in response to salinity without changing cellular structure or metabolism may be explained by the fact that Pro has a zwitterion property, which is known to be important in the osmoregulation of stressed tissues (Goyal & Asthir, 2010). It may facilitate the acclimation of *C. roseus* plants to salt stress. Comparable outcomes were found for several therapeutic plants, including moringa (Azeem et al., 2023), chamomile (Banerjee & Roychoudhury, 2017), basak (Obaidullah et al., 2022), and neem (Jahan et al., 2018).

The results of this study indicate that the accumulation of Pro in response to low (4 dS/m) and moderate (8 dS/m) salt concentrations may be a key component in salt tolerance. Nevertheless, under conditions of higher salinity (12 dS/m), hyperaccumulation of Pro was observed at 90 and 120 days in contrast to moderate salinity, indicating impaired growth performance and increased damage in response to salt stress. It has been suggested that the primary cause of the hyperaccumulation of Pro in leaf tissues following high salinity treatment may be leaf damage or a symptom of salt stress. Moreover, Pro builds up in leaves to preserve turgor and Chl concentrations, shielding plant photosynthesis from salt stress (Hnilickova et al., 2021).

Our findings also concur with Aldesuquy et al. (2012), who found that soil soaked in seawater greatly escalated the overall number of soluble sugars. Several researchers have also reported that starch and sugar buildup was exacerbated by salt stress (Sadak, 2019; Sen et al., 2022). Under salt stress, sugar buildup prevents structural and functional alterations of soluble proteins' membrane degradation (Sen et al., 2022).

Alternations of Oxidative Stress Indicators and Antioxidant Defense System under Seawater Stress

Plants suffer oxidative damage when severe salt stress disrupts the electron transport path. The extra energy released during electrochemical reactions might produce more ROS (such as H_2O_2) through the Mehler reaction. Damage markers such as H_2O_2, EL, and MDA are associated with a series of free radical production events that can impact macromolecules and cellular structures. In the current study, *C. roseus* leaves' H_2O_2 and MDA levels significantly increased due to saltwater stress (Figures 4a and 4b). The MDA, H_2O_2 concentration, and EL in *C. roseus* increased more steeply with severe salinity (12 dS/m) and extended exposure time (120 days) in comparison to low and moderate salinities (4–8 dS/m). The findings indicate that *C. roseus* can protect membranes from salt-induced damage up to a particular salinity level (Figures 4a–4c). Increased H_2O_2 concentrations are directly associated with membrane and pigment deterioration, which turnover-reduces the photosynthetic apparatus and produces more radicals.

However, H_2O_2 also functions as a signaling molecule for stress tolerance (Mehmood et al., 2023; Saleem et al., 2022). H_2O_2 appears to have been used in low- and moderate-salinity states to identify and manage saltwater stress, which helped to activate antioxidant molecules (phenol and flavonoids) and high antioxidant capacity (DPPH, $ABTS^+$) (Table 4; Figure 4). Significantly intact membrane permeability (EL) of the plant, elevated quantities of stress-adaptive compounds (pro and soluble sugars), and minimal plant DW loss at this salinity all suggest that H_2O_2 has a favorable role in *C. roseus*'s ability to withstand salt at low and moderate salinities (4–8 dS/m). Elevated MDA and EL levels indicate that the cytotoxic levels of Na^+ caused ROS to surpass the plants' threshold limit, damaging membranes and cell structure when salt stress reached high salinity (12 dS/m). These findings align with earlier research conducted in *V. negundo* (S. Das et al., 2024) and *Salvadora persica* (Rangani et al., 2016). It could cause the plant's delayed growth and noticeable biomass loss in high-salt conditions.

When the plant underwent salinity stress, it activated both enzymatic and non-enzymatic antioxidants, which scavenged ROS and reduced oxidative stress and plant cell damage. According to the current study, *C. roseus* responded to salinity stress by increasing their APX and CAT activities (Figures 4d and 4e), as reported in other species such as *V. negundo* (S. Das et al., 2024), *Moringa oleifera* (Azeem et al., 2023), *Solanum lycopersicum* (Islam et al., 2023), *Oryza sativa* (Roy et al., 2019), *Brassica juncea* (Ahmad et al., 2015), and *Glycine max* (Weisany et al., 2012). APX normally uses ascorbate to convert H_2O_2 into water in the chloroplast, whereas CAT usually works in the cytoplasm. As ROS levels rose, antioxidant enzyme activity in the plant progressively increased (Apel & Hirt, 2004). At low and moderate salinities, increased CAT activity and stable APX

levels show a balanced regulation of ROS with little membrane damage (unaffected EL). *C. roseus* apparently regulates the amount of ROS in the cytoplasm and chloroplasts at a low energy cost. According to other data, H_2O_2 was generated in the chloroplast but remained in the cytoplasm due to altered APX but elevated CAT activity (Azeem et al., 2023).

However, the activities of CAT and APX significantly increase at higher salinities, indicating a major ROS burst across the cell and advocating for robust protection at the cytoplasmic and intracellular levels. As a result, managing oxidative stress was energy-intensive and significantly affected biomass and growth (Tables 1, 2, and 4; Figure 1). According to the current study, higher H_2O_2, MDA, and EL levels, as well as the strong correlation between those levels and higher antioxidant enzyme activity, are in line with the antioxidative responses of other salt-tolerant plants (Abogadallah, 2010; Subudhi & Baisakh, 2011).

Catharanthus roseus employs a well-differentiated defense mechanism consisting of antioxidant enzymes and powerful antioxidant chemical substances to protect the oxidative balance of cells. Total phenols and flavonoids are examples of antioxidant molecules that actively absorb free radicals; nevertheless, the quantity and composition of these molecules are strongly proportional to the degree of applied stress (de Abreu & Mazzafera, 2005). The levels of TPC and TFC ascended linearly with salinity (Table 4). Similarly, increasing salinity increased antioxidant capacity as measured by DPPH and $ABTS^+$, consistent with earlier research (Bekka et al., 2022). A lesser increase in antioxidant molecules and their activity is indicative of a regulatory response at low and moderate salinities. These molecules are notably more active and numerous, which eventually causes a steep drop in growth and implies a perceived need to respond rapidly to an oxidative burst in extremely high salinity. According to reports from *M. oleifera* and other Algerian medicinal plants (Djeridane et al., 2006; Meireles et al., 2020), these elements are significantly correlated with considerably greater radical scavenging (DPPH and $ABTS^+$) capacities. It suggests that these components play a major role in *C. roseus*'s antioxidant defense.

Mineral Ion Homeostasis in Response to Seawater Stress

The effects of seawater stress on mineral absorption and transport are expected to substantially impact plants' capacity to survive salt (Taïbi et al., 2016). The ion analysis conducted for this study revealed that *C. roseus* plants' roots had accumulated more Na^+ than their leaves. However, their leaves had larger amounts of K^+ and Ca^{2+} (Figures 5a, 5b, and 5c). This varied ion distribution in different organs leads to the greater K^+/Na^+ ratio in leaves compared to roots and the superior preferential transport capacities for K^+, Ca^{2+}, and Mg^{2+} over Na^+ from roots to leaves with an increasing amount of salt (Figure 5a-5d). Higher concentrations of

Na^+ create physiological drought, which prevents plants from absorbing water and upsets the osmotic balance. According to Kronzucker et al. (2013), the accumulation of Na^+ in the cytosol hinders rubisco activity during the Calvin cycle, hence adversely affecting the photosynthetic process. It is generally agreed upon that increased root Na^+ ion concentrations relative to shoots were indicative of reduced Na^+ translocation to the shoots. As a result, the plants showed an improved tolerance to salt.

The results of the current study are well supported by data from studies on melons (Sivritepe et al., 2003) and maize (Turan et al., 2010). It is also important to keep in mind that K^+ is essential for plants to withstand salinity. It is necessary in high concentrations to reduce osmotic stress in a salinity-induced stress condition. According to Bistgani et al. (2019), salt stress promotes the transfer of K^+ to the xylem, preserving the water balance and ideal K^+/Na^+ ratio in the xylem. The concentrations of Ca^{2+} and Mg^{2+} in the leaves of the seawater-stressed *C. roseus* plants were significantly higher than in the roots (Figures 5c and 5d). This finding may indicate that ion exclusion techniques facilitate the absorption of other advantageous nutrients while decreasing the inhibitory effects of Na^+. Moreover, greater Mg^{2+} concentrations in leaves may also aid in the production of Chls, the transportation of photoassimilates, and the preservation of the chloroplast ultrastructure, all essential for preserving the best possible photosynthesis in saline environments (Isayenkov & Maathuis, 2019).

Dendrogram and PCA biplot analysis clearly demonstrated that, at 8 dS/m seawater stress, physio-biochemical features, such as H_2O_2, MDA, Pro, SS, DPPH, TPhe, and RNa^+ were upregulated, whereas LNa^+, $ABTS^+$, EL, Tfla contents were negatively affected at 12 dS/m salinity (Figure 5).

CONCLUSION

According to the findings, *C. roseus* could tolerate moderate salinity (8 dS/m) by managing its physio-biochemical traits, ion toxicity, and oxidative stress; nevertheless, biomass accumulation was impaired. However, even though this plant may produce higher concentrations of some biochemical features when cultivated in highly salinized soils (12 dS/m), it is unable to overlook the notable drop in dry biomass (72%). This research implies that *C. roseus*'s antioxidant defense system might allow it to withstand salt, making it appropriate for cultivation close to coastal regions with salinities of about 8 dS/m. It would enhance Bangladesh's economy and public health while also meeting the country's expanding demand for medicinal plants. Furthermore, land in a salinity-affected area that is not currently being farmed is appropriate for producing medicinal plants. Finally, on-farm studies should be accomplished in saline-prone areas under actual field conditions prior to it being economically cultivated in saline locations.

ACKNOWLEDGMENTS

The authors greatly appreciate Bangabandhu Sheikh Mujibur Rahman Agricultural University's Research Management Wing, Bangladesh (RMW-GN 22) for providing funds and support for this experiment. They are also grateful for the assistance with their research from the Department of Agroforestry and Environment technical personnel at Bangabandhu Sheikh Mujibur Rahman Agricultural University in Bangladesh.

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