

AMELIORATIVE EFFECTS OF SALICYLIC ACID AND PLANT GROWTH-PROMOTING BACTERIA ON IONIC AND BIOCHEMICAL RESPONSES OF WHEAT UNDER SALINITY STRESS

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ABSTRACT:

Salinity stress is a major constraint, severely affecting ionic homeostasis, photosynthetic pigments, primary and secondary metabolism in wheat plants. The present study investigated the ameliorative effects of salicylic acid (SA) and salt-tolerant plant growth-promoting bacteria (PGPB) on wheat (*Triticum aestivum* L.) under NaCl-induced salinity stress. In this study, wheat seedlings were exposed to 50 and 100 mM NaCl and then treated with SA, individual PGPB isolates (B1 and B2), or their combinations. Salinity stress markedly enhanced the Na⁺/K⁺ ratio and reduced the total chlorophyll, total protein, total sugar, reducing sugar, and total phenolic content in wheat seedlings compared with the control. However, the application of SA and PGPB, either individually or in combination, mitigated the adverse effects of salinity and improved the measured physiological and biochemical attributes. Among all the treatments, the most pronounced improvement under both salinity levels was shown by the treatments having the combined application of SA with both PGPB isolates (SA + B1 + B2). It has been shown that application of SA and PGPB, either individually or in combination, lowers the Na⁺/K⁺ ratio and results in greater accumulation of chlorophyll, protein, total sugars, reducing sugars, and phenolic compounds compared with the respective salt-stressed controls. Overall, the findings suggest that the combined application of SA and salt-tolerant PGPB is a promising strategy for improving and maintaining physiological and biochemical performance in wheat under saline conditions.

KEYWORDS: Wheat; Salinity stress; Salicylic acid; PGPB; Na⁺/K⁺ ratio; Chlorophyll; Phenolic compounds.

INTRODUCTION

Among various abiotic stresses, soil salinity is a major factor that adversely affects agricultural productivity and sustainable crop production worldwide. The problem of soil salinisation is becoming more severe due to factors such as improper irrigation practices, poor drainage, limited water availability and changing climatic conditions, especially in arid and semi-arid regions (Naorem et al., 2023). In plants, excessive accumulation of salt in the rhizosphere diminishes water uptake, nutrient absorption, photosynthetic activity, membrane stability and cellular metabolic activities by generating osmotic and ionic stresses (Mishra et al., 2021; Ren et al., 2025). Wheat (*Triticum aestivum* L.) is one of the most important cereal crops growing globally and plays an important role in global food production and human nutrition. However, its growth and productivity are adversely affected by salt stress (Manhou et al., 2026). Wheat plants respond differently to salinity depending on their genotype, growth stage, severity and duration of salt exposure (Taybi et al., 2025). Long-term exposure to salt stress can disturb essential ionic balance within plant tissues. Excessive accumulation of Na⁺ can interfere with the uptake of K⁺ ions, which are involved in various cellular processes like enzyme activation, osmotic regulation, stomatal functioning, protein synthesis and maintenance of membrane potential. This results in an increase in the Na⁺/K⁺ ratio, which is widely considered an important indicator of ionic stress and salt tolerance (Wang et al., 2024). Changes in the Na⁺/K⁺ ratio under saline conditions are closely associated with alterations in the Na⁺/K⁺ ratio, highlighting the critical role of ionic homeostasis in sustaining plant growth and performance (Liang et al., 2026). Excessive accumulation of salt can also disrupt chloroplast structure, stomatal behaviour and photosynthetic electron transport, while osmotic stress can restrict gaseous exchange and carbon assimilation. As a result, salinity can degrade chlorophyll pigments, thereby decreasing the photosynthetic efficiency of plants. Therefore, chlorophyll content is considered an important physiological indicator of plant stress tolerance (Füzy et al., 2019). Comparative studies in wheat genotypes have shown that under salt stress, salt-sensitive genotypes exhibit greater reductions in chlorophyll as compared to salt-tolerant genotypes (Hussain et al., 2022; Masarmi et al., 2023). Furthermore, recent studies on wheat genotypes demonstrated that maintaining chlorophyll a, chlorophyll b and carotenoid levels under saline conditions is associated with better physiological performance in wheat (Pastuszak et al., 2022; Akram et al., 2025). Therefore, maintaining photosynthetic pigments under saline conditions is crucial for sustaining carbon assimilation, energy production and overall plant growth.

Salinity-induced ionic and osmotic stress can cause considerable alterations in primary metabolism. The process of protein metabolism can be disrupted by altering water relations, nutrient availability, enzyme activity and the oxidative status of the cell. Disruption of protein synthesis and degradation under salt stress can affect soluble protein accumulation and overall metabolic processes (Mansour et al., 2022). Maintenance of sufficient protein levels is crucial for appropriate functioning of enzymes, cellular structure and stress-responsive metabolism. Therefore, the application of beneficial microbes has been shown to improve protein accumulation in salt-stressed wheat. Under saline conditions, inoculation of *Bacillus* sp. enhanced soluble protein accumulation, limited Na^+ accumulation, and enhanced K^+/Na^+ homeostasis in wheat, therefore supporting the maintenance of cellular metabolic functions in wheat (Liu et al., 2023).

Another important adaptation of plants under salt stress is alteration in the process of carbohydrate metabolism. Soluble sugars not only function as respiratory substrates but also as carbon reserves. Therefore, they contribute to osmotic adjustment and protect cells against the adverse effects of salinity. Accumulation of soluble sugars plays an important role in maintaining cellular water homeostasis, stabilising proteins and photosynthetic machinery under stress conditions (Khanna et al., 2023). However, salinity-induced alterations in carbohydrate metabolism are strongly influenced by genotype, tissue type, developmental stage and stress severity. Recent studies in wheat have shown that salinity alters soluble sugar accumulation and other metabolites, while tolerant genotypes show more effective metabolic adjustment than sensitive ones (Farooq et al., 2024; Ibrahimova et al., 2025). Hence, changes in total and reducing sugar accumulation can be considered as important biochemical parameters for assessing osmotic adjustment and metabolic responses of wheat to salinity.

Salt stress promotes excessive generation of reactive oxygen species (ROS), which can cause oxidative damage to lipids, proteins, nucleic acids and cellular membranes. In response to oxidative stress, plants activate both enzymatic and non-enzymatic antioxidant defence mechanisms to maintain cellular redox homeostasis (Sachdev et al., 2023; Dash et al., 2025). Phenolic compounds are important non-enzymatic antioxidants that contribute to the scavenging of ROS, thereby protecting cellular components from oxidative damage. Therefore, their accumulation under adverse environmental conditions can reflect activation of secondary metabolic defence. Recent studies have shown that under saline conditions in wheat, application of plant growth-promoting treatments may enhance the accumulation of phenolic compounds and other secondary metabolic defences (Mehrabani et al., 2024; Dargiri et al., 2025). Thus, total phenolic content may serve as a useful indicator of biochemical defence mechanisms in wheat under saline stress conditions.

Nowadays, sustainable biological approaches, particularly the use of plant growth-promoting bacteria (PGPB), have gained increasing attention as promising strategies for enhancing plant performance and salt tolerance. PGPB can direct plant growth via several mechanisms, including production of indole-3-acetic acid (IAA), phosphate solubilization, siderophore production, nitrogen fixation, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, exopolysaccharide production and modulation of plant hormonal and antioxidant responses (Pérez-Montaña et al., 2025; Singh et al., 2026). Under saline conditions, these mechanisms can enhance nutrient acquisition and water relations, ion homeostasis, osmotic adjustment and enhanced protection against oxidative stress. It has been reported that salt-tolerant PGPB can improve wheat growth and maintain a lower Na^+/K^+ ratio under saline conditions, thereby suggesting its potential role in improving ionic homeostasis and salt tolerance (Wang et al., 2026). Similarly, *Bacillus* sp. inoculation has been reported to enhance wheat biomass and root development while reducing Na^+ accumulation and enhancing the K^+/Na^+ ratio, soluble sugars and soluble proteins under salt stress (Ji et al., 2022). Application of plant growth-promoting bacteria can enhance photosynthetic activity and the accumulation of secondary metabolites, including phenolic compounds, in wheat under saline conditions (Khan et al., 2022; Jakubowska et al., 2025). Such responses suggest that beneficial microorganisms may promote both primary and secondary metabolism, thereby supporting plants in sustaining essential physiological functions and strengthening their defence against oxidative stress.

Salicylic acid (SA), an endogenous phenolic signalling molecule, is one of the important plant growth regulators involved in plant growth, development and the regulation of stress signalling. Exogenous application of SA has increasingly been recognised as a promising approach for improving plant tolerance to saline stress (Parveen et al., 2024). However, SA effectiveness varies with concentration, mode of application, plant species, genotype, and developmental stage. Appropriate concentrations of SA can stimulate protective responses, while excessive concentrations may cause adverse effects on plant growth and physiological functions (Song et al., 2023). SA's beneficial role in salt-stressed wheat is evident in its ability to regulate ionic balance by reducing Na^+ accumulation, improving K^+ status and preserving photosynthetic pigments (Shaheen et al., 2025). Recent studies have shown that exogenous application of SA can mitigate the decline in chlorophyll a and chlorophyll b caused by salinity and improve antioxidant defence in wheat seedlings exposed to NaCl stress. SA also plays an important role in maintaining osmotic balance and metabolic regulation in plants exposed to salt stress. Exogenous SA application can enhance the accumulation of soluble sugars, proteins and other osmoprotective compounds in wheat, thereby supporting cellular water balance and protecting macromolecules under salt stress (Maqsood et al., 2023). In addition, SA may improve the antioxidant defence system by regulating non-enzymatic antioxidants such as phenolic compounds. Molecular studies have further shown that SA signalling regulates osmotic balance and ROS-scavenging mechanisms in bread wheat, suggesting that its protective effects involve multiple interconnected processes rather than a single mechanism (Zheng et al., 2025).

Although PGPB and SA can individually alleviate salinity stress, their combined application may provide a more effective approach for enhancing salt tolerance. PGPB primarily influence the rhizosphere and the plant root system by regulating nutrient availability, ion uptake, phytohormone and microbial interactions, whereas SA directly modulates physiological and molecular responses to environmental stress. Therefore, PGPB and SA may act through complementary mechanisms that enhance plant protection under salinity compared with either treatment alone. Research in cereal crops provides support for this approach, indicating that co-application of rhizobacteria and SA can promote growth and help maintain chlorophyll content, plant water status and other physiological functions under saline conditions (Hajlaoui et al., 2026).

Therefore, the aim of the present study was to assess the effects of individual and combined applications of plant growth-promoting bacteria and salicylic acid on physiological and biochemical responses of wheat exposed to salt stress. Specifically, the study assessed the effects of these treatments on the Na^+/K^+ ratio, total chlorophyll, total protein, total sugars, reducing sugars and total phenolic content.

2. MATERIALS AND METHODS

2.1 Plant material and experimental design

Seeds of wheat cultivar HD 2967 (*Triticum aestivum* L.) were used for the present study and were obtained from Directorate of Wheat Research, Karnal, Haryana, India. The experiment was conducted under greenhouse conditions using plastic pots containing farm soil. For bacterial inoculation, the surface-sterilised seeds were soaked in 24 h-old cultures of *Pseudomonas* strains B1 and B2 for approximately 2–3 hr before sowing. Ten days after seed sowing, salinity was induced by different concentrations of NaCl, viz. 50 mM and 100 mM. At 11 days after sowing (DAS), a foliar spray of SA solution having 0.50 mM concentration was applied to the given treatments **T0**-Control; **T1**-NaCl (50 mM); **T2**- NaCl (50 mM) + B1 inoculation; **T3**- NaCl (50 mM) + B2 inoculation; **T4**- NaCl (50 mM) + SA; **T5**- NaCl (50 mM) + B1 inoculation + SA; **T6**- NaCl (50 mM) + B2 inoculation + SA; **T7**- NaCl (50 mM) + Coinoculation of B1 and B2; **T8**- NaCl (50 mM) + Coinoculation of B1 and B2 + SA; **T9**-NaCl (100 mM); **T10**- NaCl (100 mM) + B1 inoculation; **T11**- NaCl (100 mM) + B2 inoculation; **T12**- NaCl (100 mM) + SA; **T13**- NaCl (100 mM) + B1 inoculation + SA; **T14**- NaCl (100 mM) + B2 inoculation + SA; **T15**- NaCl (100 mM) + Coinoculation of B1 and B2; **T16**- NaCl (100 mM) + Coinoculation of B1 and B2 + SA.

2.2 Estimation of physiological and biochemical parameters

2.2.1 Determination of Na^+ and K^+ Ion Content

The Na^+ and K^+ contents of wheat plant tissues were estimated by following the method of Allen et al. (1974) and expressed as mmol kg^{-1} dry weight.

2.2.2 Estimation of chlorophyll content

Chlorophyll content was estimated using the method of Faryal et al. (2022). Approximately 500 mg of the leaf tissue sample was finely homogenized in 5 mL of ice-cold 80% (v/v) acetone using a mortar and pestle and centrifuged ($5000 \times g$) for 10 minutes. The absorbance of the resulting solution was measured at 645, 663 and 450 nm against a reagent blank.

2.2.3 Estimation of Biochemical Parameters

The total protein content was determined according to the method of Bradford (1976). About 300 mg of fresh leaf tissue was homogenized in 2 mL of 62.5 mM Tris-HCl buffer (pH 6.7) and centrifuged at $15000 \times g$ for 15 minutes. An aliquot of 20 μL of the supernatant was mixed with 150 μL of Bradford reagent and after 30 minutes of incubation, measured at 595 nm.

Total soluble sugar was quantified using the anthrone method (Jain and Guruprasad, 1989) with minor adjustments. An aliquot of 0.5 mL of ethanolic leaf extract was adjusted to 1 mL with 1 N HCl. After the mixture was boiled, 4 mL of a 0.2% anthrone solution was added and the absorbance was recorded at 595 nm using a spectrophotometer.

The reducing sugar content was estimated following the Nelson–Somogyi method (Nelson, 1944; Somogyi, 1945). Subsequently, 1 mL of the aqueous leaf extract was mixed with 1 mL of copper reagent and heated in a boiling water bath for 20 min. One mL of arsenomolybdate reagent was added after cooling and the volume was adjusted appropriately with distilled water. The absorbance of the resulting blue-coloured solution was measured at 520 nm using a spectrophotometer.

The total phenolic content was determined using the Folin–Ciocalteu method. About 100 mg of fresh wheat leaf tissue was homogenized and the resulting extract was made up to a final volume of 2 mL. After that, the extract was treated with Folin–Ciocalteu reagent, followed by the addition of sodium carbonate for colour development. The absorbance of the reaction mixture was recorded at 765 nm using a spectrophotometer.

2.3 Statistical Analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) to determine the significant differences among treatments. The significance of the treatment effect was assessed using Tukey's multiple comparison test at a significance level of $p < 0.05$. The results were expressed as mean $\pm$ standard error (SE) of the experimental observations. Statistical analysis and graphical representations were performed using GraphPad Prism.

3. RESULTS

Salinity stress markedly increased the Na^+/K^+ ratio in wheat seedlings, with increases of 48.69% and 99.51% under 50 and 100 mM NaCl, respectively, when compared with the control. Application of PGPB and SA decreased the Na^+/K^+ ratio in all treatments under both salinity levels. However, the maximum reduction was observed with the combined application of SA + B1 + B2 under 50 and 100 mM NaCl by 37.19% and 41.56%, respectively, compared with the corresponding salt-stressed controls.

A significant reduction of 31.25% and 50.97% in total chlorophyll content was observed in wheat seedlings under 50mM and 100mM NaCl respectively, compared with the control. The application of PGPB and SA resulted in an improvement in total chlorophyll content under both salinity levels. PGPB and SA treatments enhanced total chlorophyll content by 12.32–39.40% relative to the salt-stressed control (50 mM NaCl) and the maximum enhancement was observed in T8 (SA + B1 + B2). Similarly, under 100 mM NaCl, the treatments enhanced total chlorophyll content by 13.95–42.71% compared with the salt-stressed control, with the highest increase recorded in T16 (SA + B1 + B2).

Under salt stress, total protein content decreased significantly in wheat seedlings by 18.86% and 27.27% under 50 and 100 mM NaCl, respectively, compared with the control. The application of both PGPB and SA alone or in combination improved protein content under both salinity levels. At 50 mM NaCl, the highest increase in protein content was observed in T8, which was 3.67% higher than the control. Under 100 mM NaCl, the maximum protein content was observed in T16, showing only a 2.14% reduction compared with the control.

Total sugar content increased under salt stress as well as in response to the application of PGPB and SA, either individually or in combination, in all treatments. PGPB and SA treatments increased total sugar content by 13.90–38.20% over the stress control, with the maximum increase recorded in T8 (38.20%) under 50 mM NaCl stress conditions. Similarly, under 100 mM NaCl, the treatments enhanced total sugar content by 6.00–28.59% compared with the stress control, with the highest value observed in T16 (100 mM NaCl + SA + B1 + B2; $23.337 \pm 0.25 \text{ mg g}^{-1} \text{ FW}$).

Reducing sugar content increased under both salinity levels compared with the non-stressed control (T0). Under 50 mM NaCl stress, the application of PGPB and SA either individually or in combination further enhanced reducing sugar content, ranging from 9.22% to 38.58% compared with the stress control (T1). The maximum increase (38.58%) was recorded in T8, followed by T5 (30.84%) and T6 (26.37%). Under 100 mM NaCl stress, reducing sugar content increased from 3.79% to 30.33% compared with the stress control. T16 showed the largest increase in reducing sugar content, with a 30.33% increase over T9. This was followed by T13 (23.70%) and T14 (20.85%).

Total phenolic content decreased by 24.64% and 44.53% under 50 mM and 100 mM NaCl stress respectively, compared with the control. Under 50 mM NaCl stress, the application of PGPB and SA individually or in combination progressively improved total phenolic content from 10.57% to 31.21% compared with the stress control (T1). T8 showed the greatest improvement (31.21%), followed by T6 (28.66%) and T7 (24.97%). Under 100 mM NaCl stress, the treatments showed a more noticeable improvement in total phenolic content compared with the stress control (T9). The increase ranged from 14.71% to 65.61%, with the highest value recorded in T16 followed by T14 (52.42%), T13 (50.17%), and T15 (40.66%).

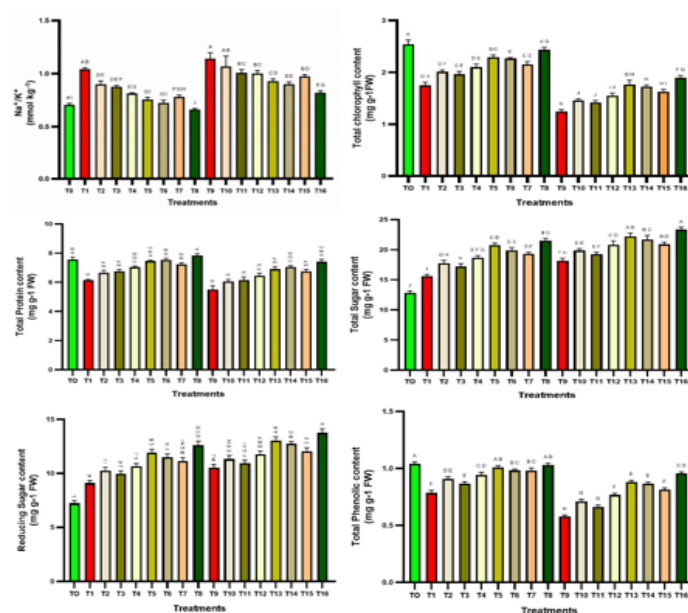


Figure 1. Effect of salicylic acid and PGPB inoculation on Na^+/K^+ ratio, Total chlorophyll content, Total protein content, Total sugar content, Reducing sugar content and Total Phenolic content of wheat seedlings under 50 mM NaCl stress (T1-T8) and (T9-T16) 100 mM NaCl stress

Values represent mean $\pm$ SD. Different letters indicate significant differences among treatments according to one-way ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Table1. Effect of salt stress and application of salicylic acid and PGPB on ionic and biochemical parameters in wheat.

Treatment	Na ⁺ /K ⁺ ratio (mmol kg ⁻¹)	Total chlorophyll (mg g ⁻¹ FW)	Total protein (mg g ⁻¹ FW)	Total sugar (mg g ⁻¹ FW)	Reducing sugar (mg g ⁻¹ FW)	Total phenolic content (mg g ⁻¹ FW)
NaCl 50 mM (T1)						
Control (T0)	0.700 $\pm$ 0.003	2.543 $\pm$ 0.08	7.572 $\pm$ 0.10	12.823 $\pm$ 0.20	7.23 $\pm$ 0.14	1.042 $\pm$ 0.016
NaCl 50mM (T1)	1.041 $\pm$ 0.010	1.748 $\pm$ 0.07	6.144 $\pm$ 0.05	15.58 $\pm$ 0.19	9.11 $\pm$ 0.14	0.785 $\pm$ 0.023
50 mM + B1 (T2)	0.862 $\pm$ 0.010	2.012 $\pm$ 0.04	6.654 $\pm$ 0.10	17.75 $\pm$ 0.29	10.27 $\pm$ 0.18	0.868 $\pm$ 0.015
50 mM + B2 (T3)	0.896 $\pm$ 0.007	1.963 $\pm$ 0.06	6.750 $\pm$ 0.09	17.123 $\pm$ 0.28	9.95 $\pm$ 0.16	0.908 $\pm$ 0.019
50 mM + SA (T4)	0.812 $\pm$ 0.003	2.104 $\pm$ 0.06	7.050 $\pm$ 0.08	18.667 $\pm$ 0.22	10.66 $\pm$ 0.17	0.944 $\pm$ 0.023
50 mM + SA + B1 (T5)	0.715 $\pm$ 0.006	2.293 $\pm$ 0.04	7.465 $\pm$ 0.08	20.773 $\pm$ 0.21	11.92 $\pm$ 0.20	0.983 $\pm$ 0.011
50 mM + SA + B2 (T6)	0.752 $\pm$ 0.001	2.269 $\pm$ 0.02	7.547 $\pm$ 0.07	19.883 $\pm$ 0.29	11.51 $\pm$ 0.19	1.010 $\pm$ 0.015
50 mM + B1 + B2 (T7)	0.780 $\pm$ 0.007	2.156 $\pm$ 0.05	7.246 $\pm$ 0.06	19.330 $\pm$ 0.17	11.13 $\pm$ 0.17	0.981 $\pm$ 0.023
50 mM + SA + B1 + B2 (T8)	0.654 $\pm$ 0.006	2.436 $\pm$ 0.05	7.850 $\pm$ 0.07	21.540 $\pm$ 0.21	12.62 $\pm$ 0.22	1.030 $\pm$ 0.017
NaCl 100 mM (T9)	1.397 $\pm$ 0.012	1.247 $\pm$ 0.04	5.507 $\pm$ 0.14	18.150 $\pm$ 0.23	10.55 $\pm$ 0.17	0.578 $\pm$ 0.013
100 mM + B1 (T10)	1.148 $\pm$ 0.009	1.462 $\pm$ 0.03	6.053 $\pm$ 0.09	19.863 $\pm$ 0.18	11.33 $\pm$ 0.17	0.663 $\pm$ 0.017
100 mM + B2 (T11)	1.202 $\pm$ 0.010	1.421 $\pm$ 0.04	6.150 $\pm$ 0.12	19.243 $\pm$ 0.20		0.712 $\pm$ 0.017

					10.95±0.16	
100 mM + SA (T12)	1.042±0.008	1.551±0.06	6.450±0.10	20.817±0.25	11.75±0.18	0.767±0.015
100 mM + SA + B1 (T13)	0.900±0.007	1.766±0.09	6.900±0.11	22.193±0.34	13.05±0.21	0.868±0.013
100 mM + SA + B2 (T14)	0.948±0.008	1.711±0.09	7.050±0.07	21.687±0.33	12.75±0.17	0.88±0.015
100 mM + B1 + B2 (T15)	0.989±0.006	1.766±0.09	6.750±0.08	20.913±0.22	12.05±0.19	0.813±0.017
100 mM + SA + B1 + B2 (T16)	0.816±0.001	1.780±0.16	7.410±0.06	23.337±0.25	13.75±0.22	0.95±0.013

4. DISCUSSION

In the present study, it was found that K^+ content decreased and Na^+ content increased in the wheat leaves under both 50 mM and 100 mM NaCl stress. This may be due to ionic imbalance (Roshdy et al., 2021) that disrupts plasma membrane integrity and causes a negative impact on the uptake of other essential nutrients (Per et al., 2017). Therefore, maintenance of a favourable Na^+/K^+ ratio is considered one of the important physiological determinants of salt tolerance in wheat (Tao et al., 2021). Similarly, in several studies, it has been reported that salt-tolerant wheat genotypes maintain a comparatively lower Na^+/K^+ ratio through restricted Na^+ accumulation and better K^+ retention under salinity (Shamaya et al., 2017; Wang et al., 2025; Shabala et al., 2026). Inoculation of PGPB in salt-stressed wheat maintains ionic balance by improving K^+ and Ca^{2+} levels and sustaining a favourable K^+/Na^+ ratio, while also strengthening antioxidant defence and reducing oxidative stress (Kumawat et al., 2023). The effectiveness of PGPB in improving the rhizospheric environment and nutrient availability under saline conditions has also been demonstrated in wheat inoculated with *Pseudomonas* and *Azotobacter* strains (Khan et al., 2025). The beneficial effect of SA observed in the present study is consistent with the established role of SA in regulating ion homeostasis under salinity. Exogenous application of SA not only improved K^+/Na^+ homeostasis but also reduced the detrimental effects of salt stress by improving photosynthetic pigments, nutrient acquisition and osmolyte accumulation in bread wheat (Abdi et al., 2022). The most pronounced improvement in ionic balance in the present study was observed when SA was combined with both PGPB isolates. The combined application of stress-mitigating agents has previously been shown to provide greater protection than individual treatments in wheat (Ali et al., 2023).

Salt stress significantly decreased the total chlorophyll content in wheat seedlings, and the reduction became more pronounced as the NaCl concentration increased (Duan et al., 2024). The reduction may be associated with ionic toxicity, impaired nutrient balance, chloroplast membrane damage and enhanced ROS production, which collectively interfere with chlorophyll stability and photosynthetic activity (Tripathi et al., 2025). The combined application of SA with both PGPB isolates resulted in the highest recovery of chlorophyll under both salinity levels, suggesting a complementary effect of these treatments in maintaining photosynthetic pigments under salt stress, consistent with previous findings in wheat (Talaat et al., 2022; Ali et al., 2023; Hafeez et al., 2024).

Salinity reduced protein content in wheat seedlings, with a more pronounced reduction at higher NaCl concentrations. However, this decline may be attributed to osmotic and ionic imbalance caused by salinity, which interferes with protein synthesis and enhances protein degradation (Patwa et al., 2024). Application of SA and PGPB partially alleviated this salt-induced decline, indicating their protective role in maintaining protein metabolism under stress (Nigam et al., 2026). Inoculation with salt-tolerant PGPB has previously been shown to improve protein accumulation and physiological performance of wheat under saline conditions (Nawaz et al., 2020; Fouad et al., 2026). SA-mediated improvement in protein metabolism has also been reported in salt-stressed wheat, where SA altered the abundance of proteins involved in stress defence, energy metabolism and photosynthesis (Talaat, 2021; Liu et al., 2022).

Salinity stress increased total sugar and reducing sugar accumulation in wheat seedlings, suggesting that increased carbohydrate accumulation may support osmotic adjustment under saline conditions (Seleiman et al., 2022). The further increase in total sugar content in plants treated with SA and PGPB suggests improved carbohydrate adjustment (Yaghoubi Khanghahi et al., 2022). Salt-tolerant PGPB have been reported to enhance total soluble sugar accumulation in wheat under saline conditions, which was associated with improved plant growth and stress tolerance (Upadhyay et al., 2015). Similarly, inoculation with *Serratia marcescens* significantly improved soluble sugar accumulation in salt-stressed wheat, indicating the involvement of bacterial inoculation in osmotic adjustment (Kulkova et al., 2024). Enhancement of soluble sugars has also been associated with alleviation of osmotic stress and improved K⁺/Na⁺ balance in wheat when treated with salt tolerant PGPB (Egamberdieva et al., 2019).

Increased total phenolic content in wheat seedlings under salinity stress, indicating an enhanced buildup of phenolic compounds as part of the plant's defence response to salt-induced oxidative stress. This has been reported under salinity stress; increased accumulation of phenols in wheat is associated with the maintenance of antioxidant capacity and protection against oxidative damage (Hajam et al., 2023). The increase in phenolic content after the application of SA and PGPB suggests that these treatments enhanced phenolic biosynthesis and strengthened the plant's secondary defence response under saline conditions (Gupta et al., 2022). The application of SA and PGPB further enhanced phenolic accumulation, suggesting stimulation of the secondary antioxidant defence system. It has been reported that SA increase phenolic and flavonoid accumulation in salt-stressed wheat, accompanied by enhanced phenylalanine ammonia-lyase (PAL) and antioxidant-related responses (Karadağ and Yücel, 2017).

5. CONCLUSION

The present study demonstrated that salinity negatively affects wheat's morpho-physiological and biochemical performance, with greater effects observed at higher NaCl concentrations. However, the adverse effects of salinity were significantly reduced by the application of salicylic acid and salt-tolerant PGPB, either individually or in combination. The combined treatment of SA along with both PGPB isolates (T8 and T16) exhibited the greatest overall effectiveness in alleviating salinity-induced alterations in growth, photosynthetic pigments, biochemical metabolism, antioxidant defence and ionic homeostasis. Overall, the findings suggest that combined application of SA with salt-tolerant PGPB offers a promising and sustainable approach for improving wheat growth and physiological performance under salinity stress.

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8. CONFLICT OF INTEREST

There is no conflict of interest.

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