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Enhancing Salt Tolerance in *Oryza sativa* through Plant Growth-Promoting Rhizobacteria via Preferential Upregulation of Plant Salt-Responsive Genes

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Abstract

Global climate change poses a major threat to food security by reducing crop productivity, particularly through soil salinization. Plant growth-promoting rhizobacteria (PGPR) offer a climate-smart and sustainable solution to mitigate salinity stress and enhance crop yield. This study investigated four potent endophytic PGPR: *Enterobacter cloacae*, *Achromobacter xylosoxidans*, *Bacillus aryabhattai*, and *Stenotrophomonas pavanii*, previously isolated from rice endophytes grown in coastal agricultural lands of Bangladesh. These strains were screened for plant growth-promoting traits and tested on the salt-sensitive rice cultivar BRRI-28 under 200 mM NaCl stress. PGPR-treated plants exhibited higher chlorophyll, carbohydrate, and protein levels, along with increased proline accumulation, indicating improved photosynthetic and metabolic activity. Reduced malondialdehyde (MDA) levels indicated enhanced membrane stability. Gene expression analysis revealed upregulation of salt-tolerance genes (GIG, BZ8, SOS1), while eEF-1α expression remained stable. These findings demonstrate that PGPR-mediated enhancement of salt tolerance in *Oryza sativa* is associated with the upregulation of key salt-responsive genes, consistent with a targeted plant–microbe interaction that may contribute to improved salinity tolerance.

Introduction

Rice (*Oryza sativa*) is the primary staple for a large proportion of the world's population, yet many high-production agroecosystems are increasingly threatened by soil salinization driven by climate change and sea-level rise. Coastal regions are especially vulnerable, where saltwater intrusion and progressive land degradation undermine sustainable rice cultivation and food security (Sultan et al. 2023, Newton et al. 2024). Globally, salt-affected soils represent a major constraint on agriculture; the FAO Global

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Map of Salt-Affected Soils highlights the broad extent of salinity-impacted land and its implications for crop production (FAO 2021). In rice and other crops, salinity can substantially reduce growth and yield by imposing combined osmotic and ionic stress and by triggering secondary oxidative damage, ultimately limiting productivity in sensitive cultivars (Tavakkoli et al. 2010, Huang et al. 2019, Kumar et al. 2020).

At the physiological level, excessive Na^+ and Cl^- disrupt water uptake and nutrient acquisition, impair photosynthesis and metabolism, and accelerate the formation of reactive oxygen species (ROS) that damage membranes and macromolecules (Tavakkoli et al. 2010, Huang et al. 2019, Kumar et al. 2020). Plants respond through coordinated mechanisms that include osmotic adjustment (e.g., compatible solute accumulation), reinforcement of antioxidant systems, and maintenance of ion homeostasis. However, such responses are often insufficient in salt-sensitive rice varieties, and conventional management approaches may be limited by cost, environmental impacts, or inconsistent performance under variable field conditions.

Plant growth-promoting rhizobacteria (PGPR) provide a promising climate-smart strategy to enhance plant resilience under salinity while reducing reliance on chemical inputs. PGPR can promote growth directly through phytohormone modulation, nutrient mobilization, and nitrogen fixation, and indirectly through improved stress buffering and plant defense stimulation (Kumar et al. 2020, Olenska et al. 2020). Under saline conditions, PGPR have been reported to support plant performance by strengthening ionic homeostasis, improving root function, and enhancing tolerance to oxidative stress (Ilangumaran and Smith 2017, Farhangi-Abriz et al. 2020). Among PGPR, endophytes—bacteria that colonize internal plant tissues without causing disease—are particularly attractive because intimate association with the host can support more stable functional interactions under fluctuating environmental conditions (Haidar et al. 2018, Muhammad et al. 2024). Nevertheless, PGPR efficacy can vary with soil type and environmental context, emphasizing the importance of selecting strains adapted to local agroecosystems and validating performance in target crops.

PGPR-mediated salt tolerance involves not only improved nutrition and hormonal regulation but also activation of host stress-responsive pathways. In rice, salinity tolerance is closely associated with ion homeostasis and stress-related gene regulation, including genes involved in Na^+ transport and transcriptional responses under stress. PGPR have been shown to influence gene expression profiles in rice during salinity exposure, supporting the concept that beneficial microbes can activate plant adaptive programs rather than merely reducing visible stress symptoms (Nautiyal et al. 2013).

Jhuma et al. previously isolated salt-tolerant endophytic PGPR from rice cultivated in salinity-prone coastal Bangladesh and characterized their key plant growth-promoting traits. (Jhuma et al. 2021). Building on that foundation, the present study evaluates four potent endophytic strains-*Enterobacter cloacae*, *Achromobacter xylosoxidans*, *Bacillus aryabhattai*, and *Stenotrophomonas pavanii*-for their ability to improve salinity tolerance in the salt-sensitive rice cultivar BRRI dhan-28. Plant growth, physiological and biochemical

stress indicators, and expression of selected salt-responsive genes (*SOS1*, *BZ8*, and *GIG*) were assessed under 200 mM NaCl stress. (Kumar et al. 2020, Jhuma et al. 2021). Overall, this research offers a cost-effective, environmentally friendly alternative to chemical fertilizers while providing insights into microbe-plant-soil interactions that support climate-resilient agriculture.

Materials and Methods

Four endophytic PGPR: *Enterobacter cloacae*, *Achromobacter xylosoxidans*, *Bacillus aryabhattai*, and *Stenotrophomonas pavanii*— isolated from rice cultivated in coastal saline fields of Bangladesh were used (Jhuma et al. 2021). Seeds of *Oryza sativa* cv. BRRI dhan-28 was obtained from the Bangladesh Rice Research Institute (BRRI).

To break dormancy, seeds were incubated at 56°C for 24 hrs, cooled at room temperature for 1 hr, soaked in autoclaved deionized water, and incubated at 37°C for 48-72 hrs until germination. Germination percentage was calculated as:

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

Uniform germinated seedlings were established hydroponically using a floating platform (Styrofoam sheet fitted with nylon mesh). Seedlings were grown in Yoshida nutrient solution (Yoshida et al. 1976), with pH monitored and adjusted as needed, and the solution replaced every 3-4 days. After 15 days, healthy seedlings were transplanted into pots for greenhouse experiments.

Agricultural soil was collected from the Botanical Garden, University of Dhaka, autoclaved, air-dried, and distributed into plastic pots (2 kg per pot). Biofertilizer formulations containing each PGPR strain were prepared following Bureau of Indian Standards (BIS) guidelines (Bhardwaj et al. 2014) and mixed into soil as per the formulation protocol. Transplanted seedlings were maintained in a greenhouse (Curzon Hall premises, University of Dhaka) at approximately 28°C and ~70% relative humidity under natural daylight. Pots were watered regularly with autoclaved deionized water to maintain soil moisture. The upper soil surface was gently loosened weekly using a sterile spatula to maintain aeration and minimize surface compaction, as soil compaction can restrict root growth and reduce water and nutrient uptake (Unger and Kaspar 1994).

To induce salinity stress, a 200 mM NaCl solution was applied in a single step to designated treatment groups 45 days after planting. Plants were maintained for 25 days following salt application prior to phenotypic, biochemical, and molecular assessments.

Treatments were arranged in a factorial structure with two salinity conditions: 0 mM NaCl control and 200 mM NaCl stress, combined with five inoculation treatments: uninoculated control and four PGPR strains. (uninoculated control and four PGPR strains). For each treatment, three pots were used, with each pot containing five plants (a total of 15 plants per treatment). Pots were distributed across the greenhouse to minimize positional effects.

For growth traits, measurements were recorded from plants within each pot and summarized at the pot level. For biochemical assays and gene expression, leaf tissue was collected from plants within each pot (with each pot considered a biological replicate), immediately processed or stored appropriately as described below. Data are presented as mean ± SD.

At 25 days after salt application, root length, shoot length, and leaf length were measured using a ruler. Dry biomass was determined by oven-drying plant tissues at 70°C for 48 h, or until a constant weight was achieved, followed by measurement of the dry weight (g).

Unless otherwise stated, physiological and biochemical assays were performed using approximately 100 mg fresh leaf tissue per replicate. All spectrophotometric measurements were blank corrected using the corresponding extraction solvent and/or reagent blank. Quantification for colorimetric assays was performed using freshly prepared standard curves, and results were normalized to fresh weight (FW).

Leaf tissue was homogenized in 80% (v/v) acetone, clarified by centrifugation at 3,000 × g for 10 min at 4°C, and absorbance was measured at 645 nm and 663 nm against an 80% acetone blank. Chlorophyll a, chlorophyll b, and total chlorophyll contents were calculated according to Inskeep and Bloom (1985) (Inskeep and Bloom 1985), using absorbance values at 645 and 663 nm, and expressed as μg g^{-1} fresh weight (FW) after normalization to extraction volume and tissue mass. Leaf tissue was hydrolyzed in 2.5 N HCl in a boiling water bath for 10 min, cooled, and total carbohydrate was quantified by the anthrone method (Yemm and Willis 1954). Absorbance was measured at 620 nm. Total carbohydrate concentration was determined from a glucose standard curve, and values were normalized to tissue fresh weight and expressed as mg/g FW.

IAA was extracted from leaf tissue using 80% cold methanol and quantified colorimetrically following Gordon and Weber (1951). Absorbance was measured at 530 nm against a reagent blank. IAA content was calculated from an indole-3-acetic acid standard curve and expressed as μg/g FW.

Free proline was determined following Bates et al. (1973). Tissue was homogenized in 3% sulfosalicylic acid, clarified by centrifugation, and proline was quantified colorimetrically by measuring absorbance at 520 nm. Proline content was calculated from an L-proline standard curve and expressed as μmol/g FW.

Malondialdehyde (MDA) was measured as an indicator of lipid peroxidation using a thiobarbituric acid reactive substances (TBARS) approach (Baryla et al. 2000, Madhava 2000). Tissue was homogenized in 0.1% TCA, clarified by centrifugation, reacted with TBA reagent, heated, cooled, and absorbance was recorded at 532 nm with turbidity correction at 600 nm. MDA concentration was calculated using:

$$\mathrm{MDA} = \frac{(A_{532} - A_{600})}{155\ \mathrm{mM^{-1}cm^{-1}}}$$

and expressed as nmol/g FW after normalization to extraction volume and tissue mass.

Protein was extracted in buffer containing 0.2% Triton X-114, 0.2 M PBS (pH 7.8), and 1 mM EDTA, clarified by centrifugation, and quantified by the Bradford assay (Bradford 1976). Absorbance was measured at 595 nm. Total soluble protein content was calculated from a bovine serum albumin (BSA) standard curve and expressed as mg/g FW.

Total RNA was extracted from frozen leaf tissue using the FavorPrep™ Plant Total RNA Mini Kit (Favorgen Biotech Corp.) according to the manufacturer's protocol. cDNA was synthesized using the ProtoScript® II First Strand cDNA Synthesis Kit (New England Biolabs, USA) with Oligo d(T)23 VN primers.

Expression of salt-responsive genes (*GIG*, *SOS1*, and *BZ8*) was assessed by semi-quantitative RT-PCR using gene-specific primers (Table 1). The housekeeping gene *eEF-1α* was used as an internal reference (Jain et al. 2006, Nautiyal et al. 2013). PCR products were resolved on 1.5% agarose gels, visualized under UV, and gel images were captured using a gel documentation system (CLEAVER Scientific Ltd, UK). Band intensities were quantified using ImageJ. For each target gene, integrated band density was normalized against the corresponding eEF-1α band from the same cDNA sample to control for variation in template input. Because PCR product sizes differed among target genes, densitometric comparisons were made within each gene across treatments using the same primer pair and amplicon size. Therefore, fold-change values represent treatment-associated changes for each individual gene and were not used to compare absolute expression levels among SOS1, BZ8, and GIG.

Table 1. Primers used for semi-quantitative RT-PCR analysis of salt-responsive genes in rice.

Target gene	Primer	Sequence (5′–3′)	Amplicon size (bp)	Annealing temperature (°C)	Reference
BZ8	Forward	TATGCATTCCGGTTACAGCA	1250	59	Nautiyal et al. (2013)
	Reverse	TACAGCATCAGTCGCCAGAC			
GIG	Forward	GTCCTGTGCTTCAATGGACC	680	59	Nautiyal et al. (2013)
	Reverse	TCCTCAATGGGAGGTTCATC			
SOS1	Forward	TCTAGTCGTTGCCAGGCTTT	800	59	Nautiyal et al. (2013)
	Reverse	TCATTGATCATGCTCCCGTA			
eEF-1α	Forward	TTCACTCTTGGTGTGAAGCAGAT	103	62.7	Jain et al. (2006)
	Reverse	GACTTCCTTCACGATTTCATCGTAA			

Results and Discussion

A total of 155 visually healthy BRRI dhan-28 seeds were selected for germination. Following dormancy breaking (56°C, 24 hrs) and incubation, 123 seeds germinated within 72 hrs, corresponding to an 79.4% germination rate. Seedlings were established hydroponically, and 95% of germinated seedlings survived following transfer and acclimatization prior to transplantation into pots.

Plant performance was evaluated under non-saline conditions and following exposure to 200 mM NaCl. Visual comparisons indicated improved vigor in PGPR-inoculated plants relative to uninoculated controls, particularly under salinity stress (Fig. 1A-B).

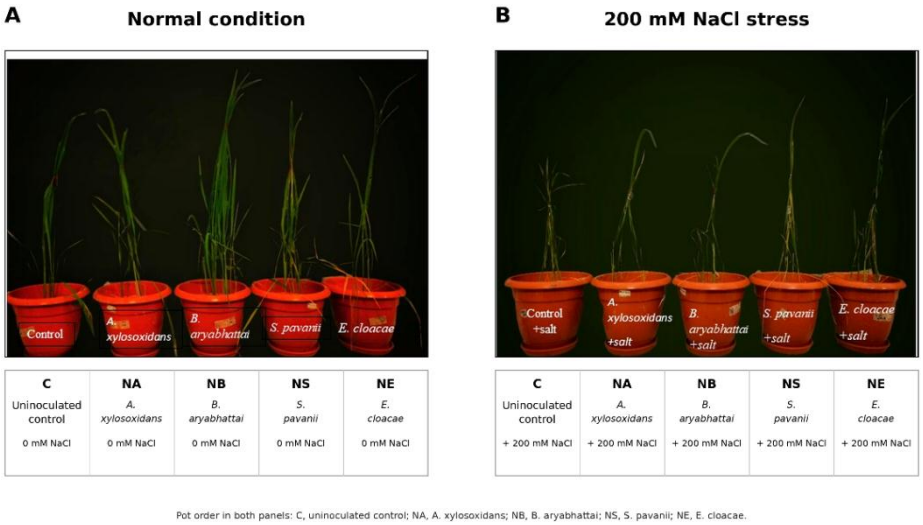

Fig. 1. Visual comparison of BRRI dhan-28 rice plants under normal conditions (A) and 200 mM NaCl stress (B) in autoclaved soil. Photographs were taken 25 days after salt application. Pots are arranged from left to right as follows: uninoculated control (C), Achromobacter xylosoxidans (NA), Bacillus aryabhattai (NB), Stenotrophomonas pavanii (NS), and Enterobacter cloacae (NE). The figure is presented as a qualitative visual comparison. Photographs were taken 25 days after salt application.

Under 200 mM NaCl, uninoculated plants showed reduced root, shoot/stem, and leaf lengths compared with the non-saline control (Fig. 2A). Among the measured length parameters, shoot/stem length showed an approximately 31% reduction under salt stress. In contrast, plants treated with PGPR maintained higher growth under salinity. Among the treatments, *S. pavanii* inoculation showed the greatest improvement, with plants exhibiting 45% greater growth than the uninoculated salt-stressed control (Fig. 2A) at the 25th day after salt inoculation.

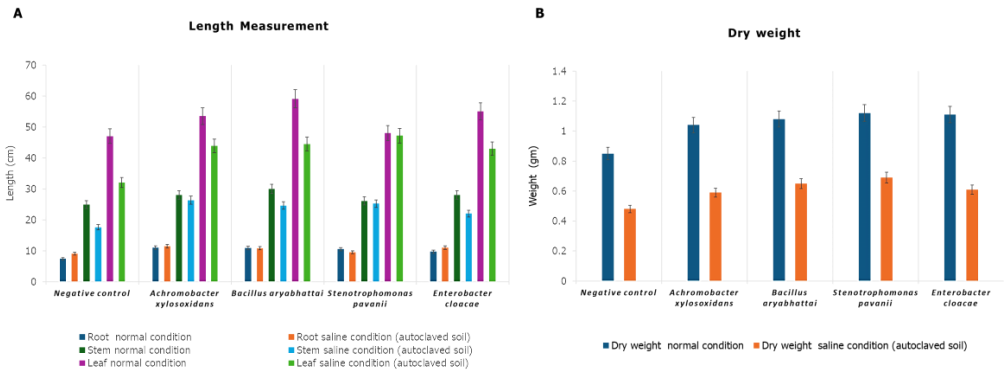

Fig. 2. Effects of PGPR inoculation on growth traits of BRRI Dhan-28 rice under normal and 200 mM NaCl stress conditions. Root, shoot, and leaf length: (A) and dry biomass, (B) are averaged as mean ± SD from n = 3 observations.

Biomass measurements followed a similar pattern. Salt stress reduced dry biomass by 44% in uninoculated plants. PGPR inoculation partially restored biomass, with dry weights higher than the salt-stressed control by 44% (*S. pavanii*), 35% (*B. aryabhattai*), 27% (*E. cloacae*), and 23% (*A. xylosoxidans*) (Fig. 2B).

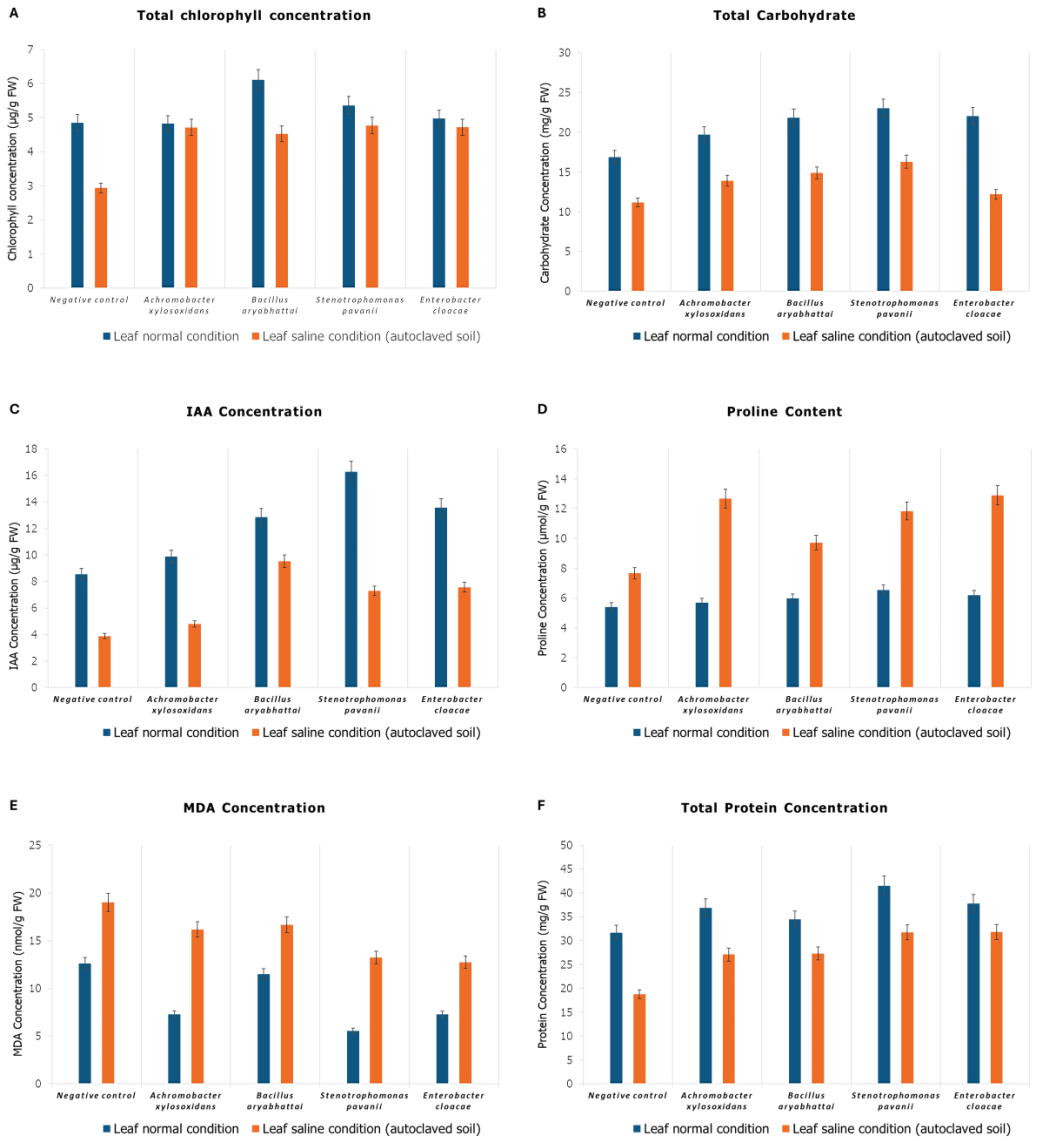

Fig. 3. Effects of PGPR inoculation on physiological and biochemical traits of BRRI dhan-28 rice under normal and 200 mM NaCl stress conditions. Total chlorophyll (A), total carbohydrate (B), indole-3-acetic acid (IAA) (C), proline (D), malondialdehyde (MDA) (E), and total soluble protein (F). Values represent mean ± SD from $n = 3$ observations.

Physiological and biochemical responses were assessed in leaf tissues collected 25 days after the single-step application of 200 mM NaCl to the soil/pot system (Fig. 3A-F). Salinity imposed clear stress in uninoculated plants, whereas PGPR inoculation mitigated these effects and maintained a more favorable metabolic status. In the uninoculated control, salinity markedly reduced total chlorophyll (Fig. 3A) and total carbohydrate (Fig. 3B), indicating compromised photosynthetic capacity and carbon metabolism. In contrast, PGPR-treated plants retained higher chlorophyll levels under salinity and showed improved carbohydrate content, with the strongest preservation generally observed for *S. pavanii* and *B. aryabhattai.* Under normal conditions, the total chlorophyll content was 4.85 μg/g FW, which declined to 2.93 μg/g FW under 200 mM NaCl-induced salt stress. However, plants inoculated with PGPR maintained chlorophyll levels of approximately ~4.7 μg/g FW, indicating a mitigation of salt-induced stress. Correspondingly, an increase in carbohydrate content was observed in inoculated plants under stress conditions. For example, *Stenotrophomonas pavanii* inoculated plant, which exhibited a carbohydrate concentration of 16.29 mg/g FW under 200 mM salt stress, compared to only 11.17 mg/g FW in untreated, salt-stressed plants.

Endogenous indole-3-acetic acid (IAA) levels declined under salinity in uninoculated plants; however, inoculated plants displayed a comparatively smaller reduction, consistent with improved hormonal status under stress (Fig. 3C).

Proline accumulation increased under salinity, with the highest levels observed in inoculated plants. Under 200 mM NaCl, proline reached 13 μmol/g FW in *E. cloacae* and *B. aryabhattai* treatments, 12 μmol/g FW in *S. pavanii,* and 10 μmol/g FW in *A. xylosoxidans,* compared with 8 μmol/g FW in the uninoculated salt-stressed control (Fig. 3D).

Lipid peroxidation, estimated by malondialdehyde (MDA), increased in the uninoculated salt-stressed control (19 nmol/g FW). PGPR inoculation reduced MDA levels under salinity, with the lowest value observed in *E. cloacae*-inoculated plants (13 nmol/g FW) (Fig. 3E).

Soluble protein concentrations increased with inoculation under saline conditions. Under 200 mM NaCl, protein levels reached 32 mg/g FW in plants inoculated with *S. pavanii* and *E. cloacae*, and 27 mg/g FW in plants inoculated with *B. aryabhattai* and *A. xylosoxidans*, compared with 19 mg/g FW in uninoculated salt-stressed plants (Fig. 3F).

Overall, the combined pattern—maintenance of chlorophyll and carbohydrate levels, partial restoration of IAA, increased proline, reduced MDA, and higher protein levels—indicates that these endophytic PGPR support metabolic stability and stress buffering in BRRI dhan-28 under severe salinity.

To elucidate the molecular basis of PGPR-mediated salinity tolerance, the expression profiles of key salt-responsive genes were analyzed under normal and salt stress conditions. This experiment was designed to determine whether bioinoculation modulates stress-associated gene expression, thereby contributing to improved plant

performance under salinity. The expression of three salt-responsive genes (*SOS1*, *BZ8*, and *GIG*) was evaluated by semi-quantitative RT-PCR, with *eEF-1α* serving as the internal reference (Fig. 4A-C). Gene-specific semi-quantitative RT-PCR reactions were performed separately for each target gene to assess treatment-associated expression patterns Densitometric analysis of the obtained bands was conducted using ImageJ software by comparing the expression levels of each respective gene under conditions without inoculum (control) with those observed following inoculum application.

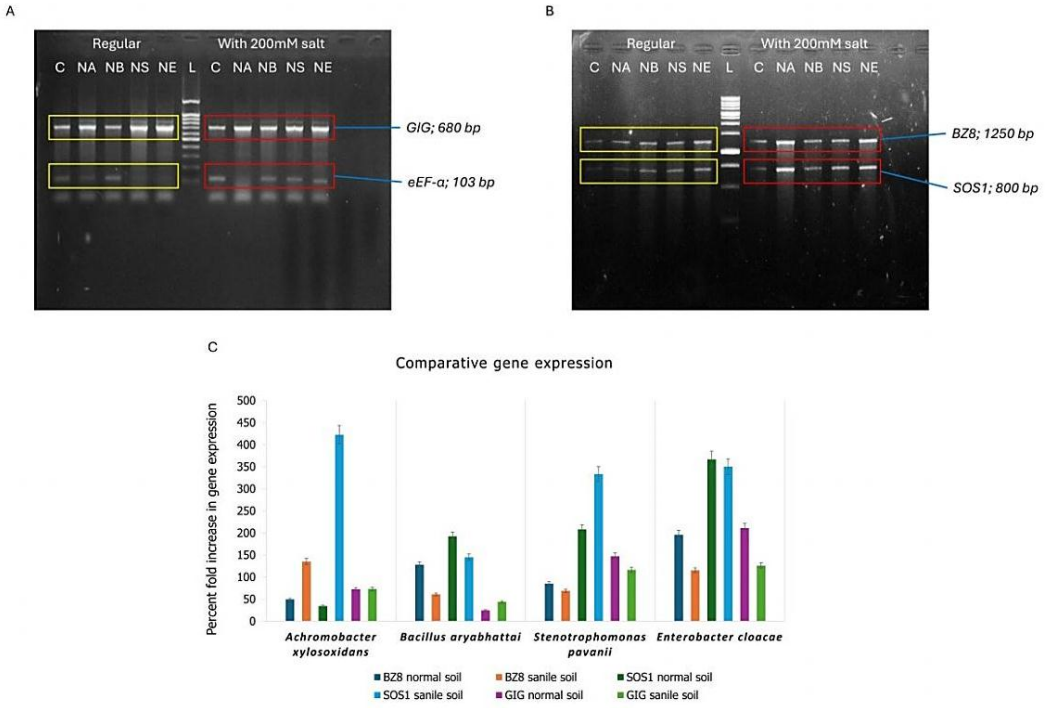

Fig. 4. Semi-quantitative RT-PCR analysis of salt-responsive gene expression in PGPR-inoculated BRRI dhan-28 rice under normal and 200 mM NaCl stress conditions. Expression of the genes was analyzed by semiquantitative reverse transcriptase polymerase chain reaction. Gene expression profiles of BZ8 and SOS1: (A) and GIG and the internal reference gene, eEF-1α, (B) are illustrated following electrophoresis on a 1.5% agarose gel. Yellow boxes indicate samples under normal conditions, while red boxes indicate samples under salt-stressed conditions. Lane designations are as follows: uninoculated control (C) plants, inoculated with *Achromobacter xylosoxidans* (NA), *Bacillus aryabhattai* (NB), *Stenotrophomonas pavanii* (NS), and *Enterobacter cloacae* (NE), and DNA ladder (L). Expected amplicon sizes of the respective genes are indicated alongside the gel. Relative fold increases in the expression of the genes of interest, namely BZ8, GIG, and SOS1, under normal and 200 mM saline conditions were calculated by comparison with uninoculated control for each inoculum treatment, as estimated by ImageJ densitometry, and are illustrated in a bar diagram (C). Target gene band intensities were quantified by ImageJ densitometry and normalized against the corresponding eEF-1α amplification obtained from the same cDNA samples. Relative expression values represent within-gene comparisons across treatments using uninoculated control as the calibrator. Because each gene was amplified and analyzed independently, fold-change values were not intended for quantitative comparison among BZ8, GIG, and SOS1.

Under saline conditions, PGPR inoculation increased the relative abundance of *SOS1* and *BZ8* transcripts compared with uninoculated salt-stressed plants. Densitometric analysis using ImageJ (performed within each gene using the same amplicon) revealed that *SOS1* expression increased by 422% (*A. xylosoxidans*), 350% (*E. cloacae*), 333% (*S. pavanii*), and 145% (*B. aryabhattai*) relative to the uninoculated salt-stressed control (Fig. 4C). Similarly, *BZ8* showed upregulation of 135, 115, 69, and 61% in the same inoculation order. The expression of *GIG* increased by approximately 116% in both *E. cloacae*- and *S. pavanii*-treated plants. In contrast, *eEF-1α* expression remained consistent across treatments, supporting its use as a normalization control. Because amplicon sizes differed among *SOS1, BZ8, GIG,* and *eEF-1α*, densitometric comparisons were interpreted within each target gene across treatments using the same amplicon, rather than as direct comparisons of absolute band intensity among different genes. The representative gel panels show selected target and reference bands; normalization was performed using the corresponding *eEF-1α* control from the same cDNA samples.

Under normal conditions, *SOS1* expression increased by 34, 366, 208, and 192% following inoculation with *A. xylosoxidans, E. cloacae, S. pavanii,* and *B. aryabhattai*, respectively, relative to the uninoculated control (Fig. 4C). Similarly, *BZ8* transcript levels were upregulated by 49, 196, 85, and 128%, respectively, in the same order of inoculation. The expression of *GIG* also increased by approximately 72, 211, 147, and 24%, respectively. These results indicate that plant–microbe interactions may play a regulatory role in the upregulation of stress-responsive gene expression.

Salinity is a major constraint to rice productivity because it imposes osmotic stress, disrupts nutrient uptake and ionic homeostasis, and promotes oxidative injury, collectively suppressing growth and biomass accumulation (Tavakkoli et al. 2010, Huang et al. 2019, Kumar et al. 2020, Rao et al. 2025). In this study, inoculation with four salt-tolerant endophytic PGPR isolated from rice plants grown in coastal agricultural lands of Bangladesh (*E. cloacae, A. xylosoxidans, B. aryabhattai*, and *S. pavanii*) mitigated the damaging effects of a severe, single-step 200 mM NaCl challenge in the salt-sensitive cultivar BRRI dhan-28. These findings extend our earlier isolation and trait characterization of these endophytes from saline-prone rice systems (Jhuma et al. 2021) and support the broader concept that PGPR can function as eco-friendly tools to improve plant performance under salinity stress (Kumar et al. 2020, Olenska et al. 2020).

A central outcome of salt stress is the deterioration of photosynthetic performance and carbon metabolism. The decline in total chlorophyll and total carbohydrate observed in uninoculated plants under salinity is consistent with salinity-induced impairment of plant metabolism and oxidative stress effects on cellular functions (Huang et al. 2019, Kumar et al. 2020). In contrast, PGPR-treated plants maintained higher chlorophyll and carbohydrate levels under salt stress, suggesting that inoculation helped preserve photosynthetic capacity and sustain carbon availability during prolonged exposure. The improved protein content in inoculated plants under salinity further supports better maintenance of metabolic activity, which commonly deteriorates under saline conditions

due to combined ionic and oxidative stress (Kumar et al. 2020, Rao et al. 2025). Collectively, these trends provide a physiological basis for the enhanced growth and biomass in inoculated plants under salinity.

Salt stress can also perturb plant hormonal balance, including auxin-mediated growth regulation. In this experiment, IAA levels were reduced under salinity in uninoculated plants but were comparatively better maintained in PGPR-treated plants. This pattern is consistent with the established ability of PGPR to support plant growth via phytohormone-related mechanisms and broader plant-microbe interactions that promote stress resilience (Ilangumaran and Smith 2017, Kumar et al. 2020, Olenska et al. 2020). Given that these isolates were previously shown to possess multiple plant growth-promoting traits (Jhuma et al. 2021), hormonal buffering likely contributed to improved growth performance under salinity.

Enhanced osmotic adjustment and membrane protection are strongly supported by the proline and MDA profiles. Proline is a key compatible solute that contributes to osmotic balance and cellular protection under abiotic stress (Bhardwaj et al. 2014, Sahoo et al. 2014, Slama et al. 2015). Under salinity, inoculated plants accumulated higher proline than uninoculated salt control, indicating stronger osmo-protective responses. In parallel, malondialdehyde (MDA), a marker of lipid peroxidation, was reduced in PGPR-treated plants compared with the salt-stressed control, suggesting improved membrane stability and diminished oxidative damage—an effect consistent with PGPR-assisted mitigation of ROS-associated injury under abiotic stress (Huang et al. 2019, Lipsa et al. 2025, Rao et al. 2025). The combined pattern of increased proline and reduced MDA therefore supports a PGPR-mediated enhancement of stress buffering capacity.

The transcriptional patterns provide a mechanistic context consistent with these physiological outcomes. SOS1 encodes a plasma membrane Na^+/H^+ antiporter central to Na^+ efflux and ionic homeostasis under salinity stress (Shi et al. 2000). The increased relative expression of SOS1 in PGPR-treated plants suggests improved engagement of ion homeostasis pathways, aligning with prior reports that PGPR can enhance salinity tolerance by helping maintain a favorable K^+/Na^+ balance and reducing ion toxicity (Sultana et al. 2020). BZ8 (a bZIP-type regulator) is commonly associated with stress-responsive regulation in rice, and its induction in inoculated plants is consistent with earlier observations that PGPR can modulate stress-related gene expression in rice during salinity exposure (Nautiyal et al. 2013). GIGANTEA (GIG) has been linked to oxidative stress response and growth regulation (Cao et al. 2006). Its increased expression in selected inoculation treatments supports the notion that PGPR may influence broader stress-adaptive regulatory networks beyond ion transport. Together, the coordinated induction of SOS1, BZ8, and GIG suggests that these endophytes not only alleviate stress symptoms but also promote host transcriptional responses associated with acclimatization.

Beyond supporting the mechanistic interpretation, these transcriptional shifts are central to the significance implied by the article title, because they indicate active plant–

microbe crosstalk under salinity stress. A particularly important outcome of this work is that salt tolerance was accompanied by elevated expression of key host salt-responsive genes, indicating an active plant–microbe crosstalk component rather than a purely nutritional or growth-promoting effect. Under 200 mM NaCl, inoculated plants showed induction of SOS1, BZ8, and GIG relative to the uninoculated salt-stressed control, while the reference gene eEF-1α remained stable, supporting a treatment-linked transcriptional response. This pattern aligns with the concept that beneficial microbes can prime or amplify stress signaling in the host through microbial elicitors and metabolites (including hormone-related modulation and other endosphere cues), thereby accelerating activation of endogenous acclimation programs during salinity exposure (Ilangumaran and Smith 2017, Kumar et al. 2020). The functional relevance of the induced targets is coherent: SOS1 encodes a plasma membrane Na^+/H^+ antiporter essential for ionic homeostasis under salinity (Shi et al. 2000), whereas BZ8 and GIG represent regulatory nodes linked to stress-responsive control and growth–stress coordination (Cao et al. 2006, Nautiyal et al. 2013). In this context, the concurrent improvements in chlorophyll, carbohydrate, and protein maintenance, proline accumulation, and reduced MDA can be interpreted as downstream outcomes of microbe-triggered activation of host stress pathways, reinforcing that these endophytes act as biological modulators of plant stress responses rather than passive amendments (Huang et al. 2019, Olenska et al. 2020, Rao et al. 2025). Taken together, the coordinated gene induction and physiological protection provide a mechanistic basis for the improved growth and biomass observed under severe salinity in inoculated plants.

Differences among strains were apparent across traits, implying functional specialization and potentially distinct modes of action. Such strain-dependent effects are expected because PGPR vary in their plant-beneficial functions, including nutrient mobilization, siderophore production, secondary metabolite secretion, and growth-promoting activities that collectively support plant performance under stress (Ali et al. 2020, Kumar et al. 2020, Sultana et al. 2021). From an application perspective, these differences could guide the selection of the best-performing strain(s) for biofertilizer development in saline rice systems.

Although the greenhouse results are promising, several steps would strengthen inference and translational relevance. Semi-quantitative RT-PCR provides directional evidence of gene induction; confirmation using quantitative expression approaches and expansion of physiological markers (e.g., tissue ion content) would further substantiate mechanistic links between inoculation, ion regulation, and stress protection (Kumar et al. 2020, Sultana 2020). Finally, because field performance can be shaped by soil complexity and environmental variability, field trials in coastal saline zones are needed to validate efficacy under realistic agronomic conditions, where PGPR-based strategies are increasingly considered part of sustainable approaches to salinity-affected agriculture (Bhardwaj et al. 2014, Sultana et al. 2021).

In conclusion, the coordinated improvements in growth, preservation of chlorophyll and carbohydrate, enhanced protein content, improved hormonal status, increased proline accumulation, reduced lipid peroxidation, and induction of key salt-responsive genes indicate that these endophytic PGPR enhance salinity tolerance in BRRI dhan-28 through complementary physiological protection and activation of host stress-response pathways (Nautiyal et al. 2013, Shi et al. 2000, Kumar et al. 2020, Sultana 2020, Jhuma et al. 2021). These locally derived endophytes therefore represent promising candidates for developing microbial interventions to support rice cultivation in salinity-affected environments in Bangladesh.

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