# ENHANCEMENT OF SALT TOLERANCE IN RICE THROUGH PLANT GROWTH-PROMOTING RHIZOBACTERIA

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**DECEMBER, 2022**

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*A thesis*
*Submitted to the Faculty of Agriculture,*
*Sher-e-Bangla Agricultural University, Dhaka*
*in partial fulfillment of the requirements*
*for the degree of*

**MASTER OF SCIENCE**
**IN**
**AGRONOMY**

**SEMESTER: JULY-DECEMBER, 2022**

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## *CERTIFICATE*

*This is to certify that the thesis entitled "**ENHANCEMENT OF SALT TOLERANCE IN RICE THROUGH PLANT GROWTH-PROMOTING RHIZOBACTERIA**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE in AGRONOMY**, embodies the result of a piece of bonafide research work carried out by **AYESHA SIDDIKA**, Registration No. **16-07028** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

*I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.*

**Dated:**
**Place:** Dhaka, Bangladesh

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# ACKNOWLEDGEMENTS

First and foremost, the author wants to express her gratitude to *Allah SWT* for His unending blessings upon her. She would like to deeply appreciate her family for their support and inspiration during the entire research journey.

The author would like to convey her profound respect and gratitude to her research supervisor, *Professor Dr. Mirza Hasanuzzaman*, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, for his academic direction in following an uncharted research path. She honestly cannot express in words how privileged she feels to have had the chance to work with him. The author feels very grateful to her supervisor for his never-ending inspiration and the thoughtful ideas he made when she was conducting the research and preparing the thesis. She feels eternally thankful to him for his assistance in helping to recognize her competence and endurance, as well as for his support in attaining her desired goal.

The author would like to appreciate her co-supervisor, Professor *Dr. Anisur Rahman*, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, for his insightful comments on the research and thesis writing. She would like to extend sincere gratitude to *Dr. Md. Mahabub Alam*, Assistant Professor, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, for his constant assistance and recommendations throughout the study process. She wants to convey her gratitude to *Farzana Nowroz*, Assistant Professor, Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, for her invaluable academic as well as mental support in solving any challenging issues.

The author would like to acknowledge Kansas State University for providing funds through USAID Foreign Assistance for Programs Overseas, for the project entitled, "Innovation Lab on Sustainable Intensification". She is also grateful to *Prof. P.V.V. Prasad*, Kansas State University, for his constructive suggestions during the study and the preparation of the manuscript.

The author would also like to extend her deepest gratefulness to *Dr. Muhammad Manjurul Karim*, Professor, Department of Microbiology, University of Dhaka, for his kind assistance in providing necessary support in this experimental work. She is also grateful to *Alfi Anjum Rashid*, MS student, Department of Microbiology, University of Dhaka, for his kind assistance with the experimental work. The author would also like to show her gratitude to *Dr. Mamunur Rahman*, Principal Scientific Officer, Farm Management Divison, Bangladesh Rice Research Institute, for his generous assistance in providing the necessary support during the experimental work.

The author wants to express how grateful she is in the deepest sense to her labmates, *Md. Rakib Hossain Raihan, Md Amir Hossain Faisal, Faria Jannat, Md. Asadul Islam, Farha Naz and Suparna Islam* for their cordial support and help throughout the research work. She would like to extend her heartfelt thankfulness to *Md. Sarwar Hosen* for his generous support with the experimental work.

The author is grateful to her sister *Suraiya Khanam* for never letting her give up in the face of adversity. She also cherishes the unwavering love and inspiration she received from her Late Father, her beloved mother and her eldest sister. The author wants to conclude by saying "Thank you" to everyone who has offered criticism since it has helped her become more energized and self-assured.

**The Author**

# ENHANCEMENT OF SALT TOLERANCE IN RICE THROUGH PLANT GROWTH-PROMOTING RHIZOBACTERIA

## ABSTRACT

The ongoing expansion of global salt-affected land is a significant factor limiting crop growth and yield, particularly for rice. This experiment explores the mitigation of salt-induced damage on rice (*Oryza sativa* L. cv BRRI dhan100) by applying plant growth-promoting rhizobacteria (PGPR) cultures. This experiment followed a completely randomized design (CRD) and experimental duration was December 2022 to May 2023. where rice seedlings, five and six weeks post-transplanting, were subjected to salt stress via two treatments with 50 and 100 mM NaCl at seven-day intervals. Bacterial cultures, comprising endophytic PGPR strains (*Bacillus subtilis* and *B. aryabhattai*) and an epiphytic PGPR strain (*B. aryabhattai*), were administered at three critical stages: during transplantation of 42-d-old seedlings, five weeks later at the vegetative stage at 35 days after transplanting (DAT), and seven weeks later at 49 DAT during panicle initiation stage. Salt stress prompted osmotic, ionic, and oxidative stress in rice plants, causing a dose-dependent decrease in relative water content, chlorophyll content, stomatal conductance, chlorophyll fluorescence, IAA concentrations, and various growth parameters. Furthermore, osmotic stress escalated the hydrogen peroxide content and proline accumulation, while ionic stress disrupted ion balance by increasing $Na^+$ and reducing $K^+$ content. Both types of stress generated reactive oxygen species, impairing the antioxidant defense system and causing oxidative damage, visible in heightened malondialdehyde levels and electrolyte leakage. PGPR treatment alleviated these negative effects by enhancing osmotic and ionic balance, demonstrated by improved water balance and reduced $Na^+$ content and $Na^+/K^+$ ratio. Additionally, PGPR fortified the antioxidative defense system in salt-exposed rice plants by increasing ascorbate and glutathione levels. The introduction of PGPR led to enhancements in yield attributes (including effective tillers per hill, panicle length, rachis per panicle, filled grains per panicle, and 1000-grain weight), consequently boosting the grain yield per hill. In conclusion, this research highlights the potential of PGPR to bolster physiological and biochemical functionality in rice, serving as an effective buffer against salt stress-induced damage

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ABA | Abscisic acid |
| ACC deaminase | 1-aminocyclopropane-1-carboxylate deaminase |
| ANOVA | Analysis of variance |
| AsA | Ascorbate |
| BS | *Bacillus subtilis* |
| BA (endo) | *B. aryabhattai* (endophyte) |
| BA (epi) | *B. aryabhattai* (epiphyte) |
| BRRI | Bangladesh Rice Research Institute |
| Car | Carotenoid |
| Chl | Chlorophyll |
| °C | Degree Celsius |
| cm | Centimeter |
| CRD | Completely Randomized Design |
| CV | Coefficient of variance |
| cv. | Cultivar |
| $CO_2$ | Carbon dioxide |
| d | Day |
| DAT | Days after transplanting |
| $dH_2O$ | Distilled water |

## LIST OF ABBREVIATIONS (Cont'd)

| | |
|---|---|
| DW | Dry weight |
| EL | Electrolyte leakage |
| EPS | Exopolysaccharides |
| *et al.* | *et alibi* (and others) |
| etc. | Etcetera |
| FAO | Food and Agriculture Organization |
| Fe | Iron |
| FW | Fresh weight |
| g | Gram |
| $g_s$ | Stomatal conductance |
| h | Hour |
| $H_2O_2$ | Hydrogen peroxide |
| ha | Hectare |
| i.e., | id est (That is) |
| IAA | Indole-3-Acetic Acid |
| K-P buffer | Potassium-phosphate buffer |
| K | Potassium |
| $K_2SO_4$ | Potassium sulphate |
| LA | Leaf area |
| m | Meter |

## LIST OF ABBREVIATIONS (Cont'd)

| | |
|---|---|
| M | Molar |
| MDA | Malondialdehyde |
| mg | Milligram |
| Mg | Magnesium |
| $MgSO_4$ | Magnesium sulphate |
| $MgCl_2$ | Magnesium chloride |
| µg | Microgram |
| ml | Milliliter |
| mM | Millimolar |
| nm | Nanometer |
| Na | Sodium |
| N | Nitrogen |
| NaCl | Sodium chloride |
| $NaNO_3$ | Sodium nitrate |
| $Na_2SO_4$ | Sodium sulphate |
| $Na_2CO_3$ | Sodium carbonate |
| $NaHCO_3$ | Sodium bicarbonate |
| $O_2^{\bullet -}$ | Superoxide radical |
| $^{\bullet}OH$ | Hydroxyl radical |
| $^1O_2$ | Singlet oxygen |

## LIST OF ABBREVIATIONS (Cont'd)

| | |
|---|---|
| $O_3$ | Ozone |
| PGPR | Plant growth-promoting rhizobacteria |
| P | Phosphorus |
| $P_n$ | Net photosynthesis |
| Pro | Proline |
| ROS | Reactive oxygen species |
| RWC | Relative water content |
| SPAD | Soil and plant analysis development |
| TBA | Thiobarbituric acid |
| TCA | Trichloroacetic acid |
| $T_r$ | Transpiration rate |
| viz. | Namely |
| Zn | Zinc |

## CHAPTER I

# INTRODUCTION

The rapid pace of urbanization and industrialization has reduced the amount of arable agricultural land available, just as the world population continues to experience significant growth (Sharma and Kumawat, 2022). As such, there is a pressing need to enhance agricultural productivity in order to meet the current food demand. However, the escalating environmental stress due to global climate change negatively impacts crop yield. This environmental stress encompasses a range of abiotic factors such as salinity, drought and waterlogging, heat stress, cold injury, light stress, UV radiation, heavy metal/metalloid stress, exposure to excessive ozone ($O_3$), and even the toxicity of plant mineral nutrients in the soil. Among these, salinity is one of the most devastating abiotic stresses to crop productivity, which is currently stagnating due to these environmental stresses (Hasanuzzaman *et al*., 2022a). Salinity is a growing global agricultural problem that renders vast areas unsuitable for crop cultivation.

Salinity refers to the excessive absorption of salts like NaCl, potassium ($K^+$), and calcium ($Ca^{2+}$) in the soils, with sodium ($Na^+$) and chloride ($Cl^-$) being the dominant ion species. High salt ion concentration in soil interferes with natural soil processes and ultimately hinders plant growth and productivity. It affects every stage of a plant's life cycle, from germination to yield, by impacting morphophysiological and biochemical processes (Roman *et al*., 2020). In a saline environment, plants generate high levels of reactive oxygen species (ROS), leading to oxidative stress (Desoky *et al*., 2020). Nevertheless, plants have intrinsic mechanisms that scavenge ROS through enhanced antioxidant defense systems (Hasanuzzaman *et al*., 2020a).

In the face of these challenges, sustainable agriculture is essential to meet global food demands and ensure future food security. Plant growth-promoting rhizobacteria (PGPR) have garnered attention in recent years for their potential to improve soil ecosystems and crop yields in stressful environments. These beneficial bacteria colonize the plant root system or rhizospheric area and stimulate growth without

negatively impacting the surrounding environment. They enhance plant growth either directly or indirectly through nitrogen ($N_2$) fixation, solubilization of essential nutrient elements like phosphorus (P), potassium (K), zinc (Zn), among others; production of phytohormones such as indole-3-acetic acid (IAA), exopolysaccharides (EPS), siderophores, 1-aminocyclopropane-1-carboxylate (ACC) deaminase and antioxidants; disease suppression through antibiotic production; bolstering plant resistance to biotic and abiotic stresses; and promoting plant-microbe symbiosis (Khan *et al*., 2017a; Verma *et al.*, 2018; Chakraborty *et al*., 2021; Dame *et al*., 2021). Their ability to reduce environmental stress on plants contributes to improved plant growth and stress tolerance. Hence, PGPR can serve as ecological engineers for climate-smart farming.

Bacteria including *Agrobacterium*, *Azospirillum*, *Arthrobacter*, *Azotobacter*, *Rhizobium*, *Bacillus*, *Erwinia*, *Bradyrhizobium*, *Burkholderia*, *Pseuodomonas*, *Achromobacter*, *Enterobacter*, *Chromobacterium*, and others induce plant tolerance to salinity and other abiotic stresses, promoting overall plant growth. For instance, *Bacillus* sp. is a notable PGPR that enhances the morphophysiological attributes of plants and aids their survival in stressful conditions. *Bacillus* sp. application results in improved plant growth, water retention, and reduced ionic toxicity, membrane damage, and electrical conductivity, thus mitigating salt-induced damage (Ji *et al*., 2022; Hasanuzzaman *et al*., 2022a). Although the beneficial effects of endophytic PGPR *B. subtilis* (Woo *et al.*, 2020; Hasanuzzaman *et al.*, 2022b), *B. aryabhattai*, and epiphytic PGPR *B. aryabhattai* (Sultana *et al.*, 2020; Sultana *et al.*, 2021), in inducing plant stress tolerance have been noted their specific roles in mitigating oxidative stress under salt stress in rice (*Oryza sativa* L.) remain understudied. Moreover, in Bangladesh, the problem of salinity in rice cultivation intensifies during the boro season due to the overuse of groundwater for irrigation. Considering the potential of these PGPRs to alleviate salt stress in rice, this experiment is designed with the following objectives:

i. Examine the impact of salt stress on the performance of boro rice.
ii. Investigate salt stress-induced oxidative damage in boro rice.
iii. Study the role of endophytic PGPR *B. subtilis*, *B. aryabhattai*, and epiphytic PGPR *B. aryabhattai* in mitigating oxidative damage in boro rice under salt stress conditions.

# CHAPTER II

# REVIEW OF LITERATURE

## 2.1 Rice for global food security

The demand and supply of food are directly tied to population growth, especially with the production of staple cereal crops like rice (*O. sativa* L.). Consumed by more than half of the world's population, rice serves as the primary food source for many. Over the past three decades, global rice production has steadily increased to meet rising demand (OECD, 2019). As a rich source of carbohydrates, rice is an annual monocot crop. Roughly 150 million hectares of agricultural land worldwide are dedicated to rice cultivation each year, producing nearly 500 million metric tons (Kumar *et al*., 2023).

While rice is a staple for nine out of ten people in Asia, it's also becoming the fastest-growing major crop in Africa, with increasing popularity in Latin America and the Caribbean. Of the 24 rice species, only two (*O. sativa* and *O. glaberrima*) are grown globally, with China and India accounting for half of the world's production and consumption (Uyeh *et al*., 2021). Although Asia produces 90% of the world's rice, demand is on the rise, a challenge exacerbated by climate change's negative impact on cultivation conditions (Bandumula, 2018; FAOSTAT, 2021).

Rice thrives in different climates and soils, with optimal conditions found in tropical and sub-tropical regions with abundant rainfall. Yet, this variability also brings diverse cultivation challenges. Despite its adaptability to irrigated conditions, rice is salt-sensitive, and salt stress can impair various morphophysiological and biochemical processes, leading to reduced growth and yield. Salt stress is particularly detrimental at the seedling establishment and reproductive stages (Korres *et al*., 2019). To meet the needs of an expanding global population, sustainable and environmentally friendly strategies for increasing rice production, particularly in salt-affected areas, are of paramount importance.

## 2.2 Abiotic stress: A major challenge for crop production

Climate change has rendered plants more vulnerable to environmental stressors, significantly disrupting their natural growth and development processes. Alongside biotic stressors, abiotic stresses severely impact plant growth and productivity, leading to negative effects on global crop production. Abiotic stresses encompass salinity, water stress (e.g., drought, and waterlogging), extreme temperature stress (e.g., heat stress, and cold injury), toxic metal/metalloid stress, elevated carbon dioxide ($CO_2$) stress, and elevated $O_3$ stress. Such stresses detrimentally impact plant cells, metabolic processes, and physiological functions, resulting in significant agroeconomic losses (Sachdev *et al*., 2021).

Given that abiotic stressors frequently occur simultaneously and are closely interrelated, their impacts on plants are particularly severe (Figure 1). One common consequence is the production of ROS, including hydrogen peroxide ($H_2O_2$), singlet oxygen ($^1O_2$), hydroxyl radicals ($OH^{\bullet}$), and superoxide anions ($O_2^{\bullet-}$). While various cellular organelles, including the chloroplast, endoplasmic reticulum, mitochondria, plasma membrane, peroxisomes, and apoplast, are capable of producing ROS, the primary sthisces are the chloroplasts, mitochondrial respiratory electron transport system, and peroxisomes (Hasanuzzaman *et al*., 2021a). Although initial excess production of ROS might not immediately disrupt cellular processes and may even be beneficial to plants, a sudden increase often serves as an alarm signal, triggering adaptive responses.

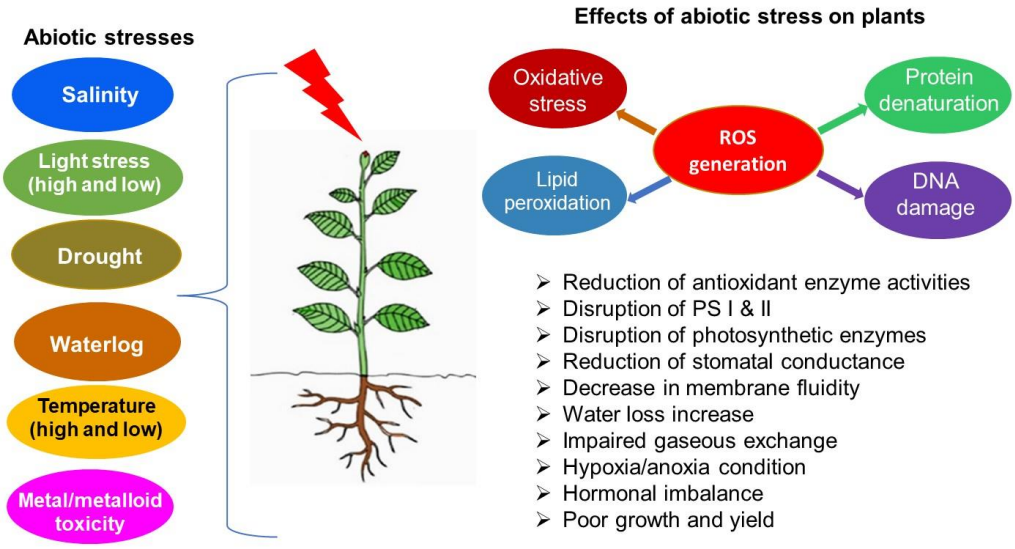


**Figure 1. Plant response under abiotic stresses** (Kumari *et al.,* 2022)

Salinity is one of the most severe abiotic stresses, causing significant annual yield loss due to its detrimental impacts on crop growth and development. For example, under 200 mM NaCl stress for 3 h at 14 days post-sowing, the growth and development of *Phaseolus vulgaris* were markedly affected (ElSayed *et al*., 2021). This stress led to a significant decrease in plant fresh weight (FW) and dry weight (DW) due to the excessive generation of lipid peroxidation and $H_2O_2$ content by 39 and 50%, respectively.

According to Parveen *et al*. (2019), *Zea mays* growth (cvs. Pearl and Malka) was compromised at 60% field capacity, leading to decreased chlorophyll (Chl) pigment activities (viz., Chl *a*, Chl *b*, Carotenoid (Car)), and a significant reduction in FW, DW, and length of shoot and root. Furthermore, different durations of waterlogging stress (viz., 3, 6, and 9 days) led to dose-dependent negative impacts on the morphophysiological parameters of *Glycine max* cv. Sohag (Hasanuzzaman *et al*., 2022b). Heat shock also inhibits crop growth by increasing leaf chlorosis, causing membrane injury, protein and enzyme denaturation, and creating water deficit conditions in plant cells. For instance, a day/night temperature of 32/20°C for 12 h significantly decreased pollen germination, increased pollen death, and reduced stigma receptivity and ovule viability in *Lens culinaris* plants (Bhardwaj *et al*., 2021).

## 2.3 Salinity stress

Salinity stress, one of the most severe abiotic stresses, severely affects crop quality and productivity worldwide. Both natural and anthropogenic activities contribute to the stress, threatening over 20% of globally cultivable lands, a situation that continues to worsen (Arora, 2019). Salinity stress negatively impacts plant growth, development, and reproduction in various ways. Plants, which rely on soil for minerals and nutrients, can experience stress from excessive soluble salts, which increase intracellular ionic concentrations and osmotic pressure (Zhao *et al*., 2021).

Excessive water-soluble salts such as sodium chloride (NaCl), sodium nitrate ($NaNO_3$), potassium sulfate ($K_2SO_4$), sodium sulfate ($Na_2SO_4$), sodium carbonates ($NaHCO_3$ and $Na_2CO_3$), calcium sulfate ($CaSO_4$), magnesium chloride ($MgCl_2$), and magnesium sulfate ($MgSO_4$) can accumulate, causing salinity or salt stress in plants (Zhao *et al*., 2021). This leads to ionic toxicity, osmotic shock, nutritional imbalance, oxidative stress, and hormonal imbalances, which can ultimately result in plant death. Besides osmotic shock and ionic stresses, salt stress triggers the formation of ROS in cells, causing severe damage to cell membranes, DNA, lipids, and enzymes (Ahanger *et al*., 2017).

Salinity can cause plant death in three primary ways. First, high salt ionic concentrations in the soil lower its water potential, changing its hydraulic conductivity and permeability. The reduced soil water potential results in water stress, causing physiological drought conditions, protein breakdown due to excessive $Na^+$ concentrations, and cell membrane instability. Salt stress not only changes plant physiology and metabolism but also affects seed germination, decreases photosynthesis and other biosynthetic processes, and inhibits growth (Nosek *et al*., 2021). The effect of salinity varies among crops. While halophytes can survive and reproduce under salt stress, glycophytes typically exhibit reduced growth and yield. Therefore, osmotic pressures and ionic toxicity together lead to secondary stresses, which impede seed germination and seedling establishment, disrupt physiological activities, and cause a reduction in growth and yield.

### 2.3.1 Salt stress affects the germination and establishment of seedlings

Germination, a critical phase in a plant's life cycle, significantly influences seedling establishment and subsequent growth, development, and reproduction of the plant. It is well established that osmotic and ionic stress caused by salt in germination media hinder seedling germination and establishment (Rahman *et al*., 2016). Salinity obstructs seed germination by interfering with key germination processes, such as imbibition, metabolic pathway activation, embryonic development, and seedling establishment. Osmotic and ionic changes induced by salinity stress hinder the germination process, inhibiting hydrolysis, causing cell membrane breakdown, and reducing enzymatic activation. Consequently, the reduced hydrolysis limits the transfer of stored food from tissue to the developing embryo, hampering seed germination and seedling establishment.

Salinity inhibits amylase activity, leading to insufficient hydrolysis or absorption of stored chemicals. However, the adverse impact of salinity on seed germination may vary, depending on the salinity level and other external conditions. Salinity significantly affects the rate and speed of germination, and establishment of rice seedlings, as it does with other crop plants (Hua-long *et al*., 2014). Numerous studies have found that salinity decreases the percentage of germination, germination index, speed of germination, mean germination time in rice, and eventually seedling establishment. Additionally, Ologundudu *et al.* (2014) discovered that eight rice cultivars with varying salinity (0–15 dS $m^{-1}$) experienced a decrease in germination percentage and speed of germination. In their research with rice plants, Rahman *et al*. (2016) found that salt stress resulted in water deficit and chlorosis in the plant, which eventually slowed plant growth.

### 2.3.2 Plant growth under salinity stress

The earliest phases of seedling establishment and vegetative growth are the most susceptible to salinity in a plant's life cycle (Rahman *et al*., 2016). Rice, in particular, is salt-sensitive, with sensitivity fluctuating based on its developmental stage. Early seedling stages are considered the most sensitive to salinity (Kumar *et al*., 2016). High

salt concentrations surrounding the roots directly cause osmotic stress in plant seedlings, impairing their water absorption capacity. This stress results in cell water loss, disrupts cell division and elongation, and triggers stomatal closure, eventually diminishing leaf area (LA), photosynthesis rate, and overall plant growth over time (Hasanuzzaman *et al*., 2021a). Moreover, a high salt content near the rhizosphere can inhibit plant growth by creating ionic stress due to an excess of $Na^+$ and $Cl^-$. Salinity disrupts ionic homeostasis by overaccumulating $Na^+$, reducing $K^+$ uptake (Rahman *et al*., 2023). The excessive influx of $Na^+$ minimizes the photosynthetic surface available for salt-affected plant growth by causing leaf chlorosis, necrosis, and early senescence of older leaves. During advanced plant life stages, salt-induced ionic and osmotic stresses, alongside a reduced photosynthesis rate, lead to oxidative stress via ROS overproduction, possibly contributing to diminished growth under salty conditions (Hasanuzzaman *et al*., 2021a). This also hampers the plant's ability to absorb several nutrients.

Various studies have demonstrated salt stress's negative impact on rice growth. Increased salinity levels (150 mM NaCl) led to reduced plant height, FW, and DW due to osmotic and ionic toxicity, and oxidative stress (Rahman *et al*., 2016). In another study, under 200 mM NaCl stress, plant growth significantly decreased due to ionic imbalance and oxidative damage (Rahman *et al*., 2023). Similarly, Kumar and Khare (2016) showed that 100 mM NaCl reduced root and shoot length, and DW of both root and shoot in both salt-sensitive and salt-tolerant rice cultivars, with a larger growth decrease in sensitive cultivars.

### 2.3.3 Plant physiological responses under salinity

Salinity detrimentally impacts plant physiology, particularly photosynthesis—a critical process for plant growth and development. Photosynthesis relies on several factors, including the photosynthetic apparatus's production, gas exchange, electron transport system, photosynthate assimilation, and various carbon metabolism-related enzymes. Thus, any damage to these components significantly hampers photosynthesis (Abideen *et al*., 2020). Under salt stress, osmotic stress reduces the photosynthetic rate by disturbing stomatal movement and facilitating excessive $Na^+$ and $Cl^-$ buildup,

potentially damaging the chloroplast's thylakoid membrane (Hasanuzzaman *et al.*, 2021a). Salinity led to a reduction in Chl *a* (23%) and Chl *b* (19%) content in twelve-day-old rice seedlings, when exposed to 150 mM NaCl for three days (Rahman *et al*., 2016). When this treatment continued for another three days, Chl *a* and Chl *b* decreased by 46% and 48%, respectively. Salinity affects plant photosynthesis by altering stomatal conductance ($g_s$), water status, transpiration rate ($T_r$), and increasing intracellular NaCl levels (Kwon *et al*., 2019). Depleted concentrations of Chl *a*, Chl *b*, and Car in rice plants resulted in a reduction in net photosynthetic rate ($P_n$), $T_r$, and $g_s$, thereby significantly hampering photosynthesis (Taj and Challabathula, 2021). Under salt stress, plants exhibit a larger Chl *a*/*b* ratio compared to stress-free conditions, leading to reduced photosynthesis. Even brief salinity exposure triggers Chl degradation, which intensifies with extended stress duration (Parvin *et al*., 2019). Rahman *et al*. (2016) observed that the rise in $Na^+$ and the $Na^+/K^+$ ratio during salt stress, in contrast to stress-free conditions, disrupted ionic balance in *O. sativa* seedlings. This also negatively influenced the Zn concentration, both in plant shoots and roots, along with a drop in $K^+$, $Ca^{2+}$, and $Mg^{2+}$ levels.

### 2.3.4 Effect on crop yield and yield parameters

According to Ramadan *et al.* (2019), salinity primarily inhibits germination and plant growth and subsequently lowers the yield, negatively affecting yield-related parameters and grain quality. Apart from impacting vegetative growth, salinity damage extends to a plant's yield and reproductive health. It substantially affects rice yield and grain quality by impeding growth, photosynthesis, and the net absorption rate. Besides the vegetative stages, salt stress also impacts rice's reproductive stage, reducing yield, yield-contributing factors, and grain quality (Noreen *et al*., 2021).

Kumar and Khare (2016) noted that 100 mM NaCl salinity stress (≈10 dS $m^{-1}$) decreased grain per panicle, filled grains percentage, 1000-grain weight, and grain yield in both sensitive and tolerant rice cultivars, with a higher yield reduction in the sensitive cultivar. They also found that salinity decreased the protein and starch levels in rice grains, compromising grain quality. In another study, Chunthaburee *et al*. (2015) found that under 25 mM NaCl stress, the harvest index and yield declined due to reduced

1000-grain weight, filled grain percentage, and panicle viability. Furthermore, according to Arsa *et al*. (2016), 2.5% NaCl stress reduced rice grain production, flavor, and total yield with increasing salinity levels.

### 2.4 Salt-induced oxidative stress and antioxidant defense system of plants

One common outcome of salt stress is the increased generation of ROS in plants. High salt stress leads to stomatal closure and a consequent decrease in the $CO_2$ available for fixation in leaf tissues. With chloroplasts exposed to excessive excitation energy and reduced capacity of the Calvin cycle to fix $CO_2$, the electron transport system in the photosynthetic process gets impaired (Rahman *et al*., 2016). Salt stress induced by 150 mM NaCl led to a time-dependent increase in oxidative stress. After three and six days of exposure to salt stress, malondialdehyde (MDA) content rose by 80% and 203%, respectively, and $H_2O_2$ concentration by 74% and 92%, respectively. Compared to the control, $O_2^{\bullet-}$ and $H_2O_2$ deposition in leaves was significantly higher after six days of stress.

Excessive ROS production during salt stress is relatively common, influenced by the plant's genotype, salinity levels, and duration of exposure. Tolerant plants produce fewer ROS, correlating with their stronger antioxidant defense. ROS serves both beneficial and detrimental roles in plants, making the maintenance of cellular equilibrium vital. They can steer plant growth and development and aid in stress adaptation. However, when ROS concentrations surge under stressful conditions, they negatively impact cellular metabolism. Nevertheless, plants possess an efficient antioxidant system that balances cellular redox potential (Duan *et al*., 2021). The enzymatic and non-enzymatic elements within plants' antioxidant defense mechanisms counteract or inhibit ROS-induced oxidative damage, thus preventing cellular harm (Dumont and Rivoal, 2019).

## 2.5 Rice and salt stress

Salt stress impacts plant biology and physiology at every growth stage, from germination to senescence. Osmotic and ionic toxicity induced by salinity results in oxidative damage and nutritional depletion in plant cells (Razzaq *et al.*, 2020). It triggers various morphological alterations in rice, including stunted root system emergence, leaf curling, chlorosis, fewer tillers per plant, diminished biomass, decreased plant height, reduced 1000-grain weight, fewer spikelets per panicle, and a greater percentage of sterile florets (Figure 2). These alterations ultimately diminish the harvest index and grain yield (Machado and Serralheiro, 2017; Razzaq *et al.*, 2020; van Zelm and Zhang, 2020).

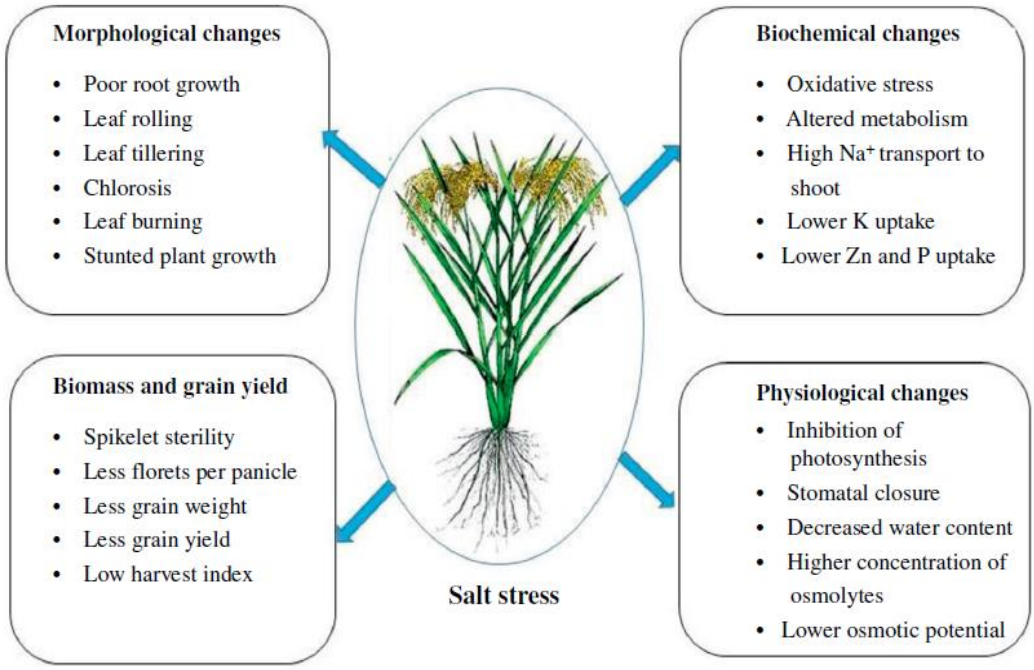


**Figure 2. A diagram depicting various impacts of salt stress on the morphophysiological, biochemical attributes and reproductive stages of rice** (Riaz *et al.,* 2019)

The primary cause of salinity-induced damage in rice plants is excessive $Na^+$ absorption, rather than osmotic or water stress, although water uptake is diminished. Upon encountering salt stress, plants swiftly detect osmotic stress, caused by a lowered water potential of the external solution, which leads to water deficit conditions. Quick stomatal closure due to osmotic stress also hinders the plant's ability to absorb $CO_2$,

thus obstructing photosynthesis (Yang and Guo, 2018). Therefore, to respond to salt stress, rice plants need to adjust their physiological and biochemical mechanisms to manage oxidative stress, nutritional balance, ionic equilibrium, and osmotic homeostasis.

### 2.6 Approaches of increasing salinity tolerance in rice

Plant salt tolerance is a complex trait. Many plant species are believed to possess innate cellular processes that foster salt tolerance. A plant that can survive and prosper in a medium with high levels of soluble salt is considered salt tolerant. If plant rhizospheres contain high salt concentrations and the plants can survive and thrive in such conditions, they are termed halophytes. Halophytes may be obligate, exhibiting few morphological and taxonomical differences, while glycophytes are believed to possess unique cellular mechanisms for salt tolerance.

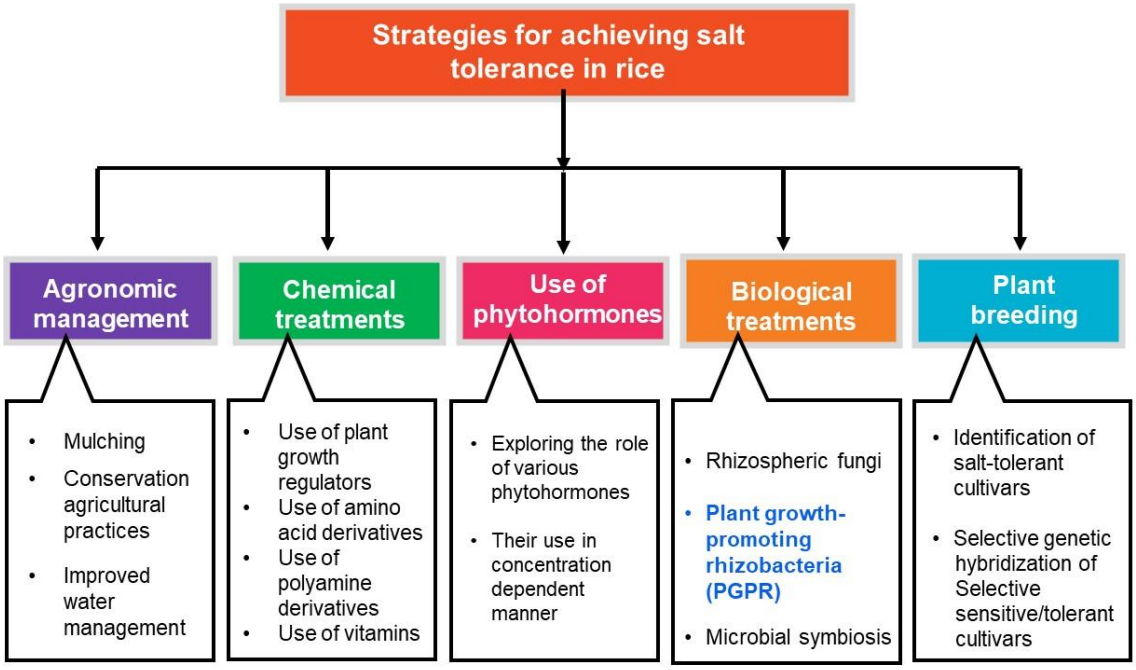


**Figure 3. Several methods to elevate salinity-tolerance of rice** (Kaur *et al.,* 2019)

Addressing production under salinity stress conditions sustainably is essential to meet consumer demand. Various tools and methods have been employed thus far to develop salt-tolerant rice strains. This includes techniques for water and soil management, as well as breeding approaches that emphasize salinity tolerance (Hoang *et al*., 2016).

The advancement of research tools like recombinant DNA technology, DNA sequencing, and microarray imaging have spurred the development of innovative strategies for enhancing rice's tolerance to salinity stress and broadened this understanding of salt stress biology (Kaur *et al*., 2019). Consequently, an integrative approach, which combines biotechnology and molecular marker techniques with conventional breeding methods, is deemed the most suitable for cultivating salt-tolerant rice.

Currently, the primary five methods used to improve salt stress resistance include breeding, marker-assisted selection, the external application of plant growth regulators, biotechnology, and genome editing (Figure 3).

Beyond these methods, the application of osmoprotectants, phytohormones, signaling molecules, polyamines, and PGPR is also increasingly recognized for their role in enhancing salt stress tolerance in rice and other crops (Hasanuzzaman *et al.*, 2021c). Table 1 outlines the implications of various approaches on enhancing the salinity tolerance of rice crops.

**Table 1.** The implications of various approaches in enhancing the salinity tolerance of rice crops

| Plant | Salinity dose | Approaches | Effects | References |
|---|---|---|---|---|
| *Oryza sativa* ssp. Indica | 150 mM NaCl | 5 µM ABA pretreatment for 48 h | Enhanced metabolic activities and antioxidant defense system, less proline (Pro) content, higher biomass accumulation | Li *et al.* (2010) |
| *O. sativa* cv. IR 651, IR29 | 6 dS $m^{-1}$ NaCl from panicle initiation to harvest | 50 µM kinetin foliar spray | Improved accumulation of sucrose and glucose content, filled grains (%), 1000 grain-weight, and total yield | Javid *et al.* (2011) |

| Plant | Salinity dose | Approaches | Effects | References |
|---|---|---|---|---|
| *O. sativa* | 0.5, 1.0, 1.5, 2.0 and 2.5 g NaCl $kg^{-1}$ soil | *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* | Decreased lipid peroxidation and superoxide dismutase activity with enhanced plant growth and development | Jha and Subramanian (2014) |
| *O. sativa* L. cv. BRRI dhan29 and BRRI dhan47 | 50 and 100 mM NaCl | 25 and 50 mM Pro | Elevated chlorophyll content, Pro and improved antioxidant enzyme activities as well as enhanced biomass build-up and yield | Bhusan *et al.* (2016) |
| *O. sativa* | 100 mM NaCl | *B. amyloliquefaciens* | Plant biomass, water content, and Pro should all be increased while reactive oxygen activity should be decreased. | Shahzad *et al.* (2016) |

### 2.7 Application of PGPR in enhancing plant resistance to salinity

Soil microorganisms represent a potential alternative method for soil rehabilitation and increasing plant tolerance to salt stress. By providing essential nutrients like N, P, K and hormones such as auxin, cytokinin, and abscisic acid (ABA), and by reducing ethylene production, symbiotic bacteria enhance plant growth and alleviate salt stress. Two types of soil microorganisms, epiphytic and endophytic bacteria, facilitate plant growth. Epiphytic bacteria, known as PGPR, are associated with the external parts of plant roots in the rhizosphere (Gerhardt *et al*., 2017). On the other hand, endophytic bacteria are beneficial microbes that reside within plant roots or interact with other organisms. To survive in high-salinity environments, salt-tolerant microorganisms and plants have co-evolved adaptive mechanisms.

## 2.8 Mechanisms of PGPR-mediated salinity tolerance in rice

Salinity stress negatively impacts plant physiology, metabolic processes, and morphology. Some plants, especially halophytes, accumulate salt in their cellular xylem and expel it through their leaves, while others have developed specialized organs, such as salt glands, that release salt. This salt is then dispersed by external forces like wind or water (Gao *et al*., 2022). Plant growth-promoting rhizobacteria in plant tissues help plants cope with salinity stress through the secretion of various compounds, ABA, IAA, ACC deaminase, and different volatile compounds. While both endophytic and epiphytic PGPR can enhance plants' stress responses, endophytes' living conditions are unique and are not influenced by the presence of other bacteria or soil pH (Santoyo *et al*., 2016).

Figure 4 illustrates how PGPR improves plant salt tolerance. PGPR enhances plant salt tolerance through several processes such as P and K solubilization, iron (Fe) chelation, atmospheric $N_2$ fixation, maintaining water status within cells, selective absorption of $K^+$ and exclusion of $Na^+$ to maintain a high $K^+/Na^+$ ratio, production of EPS to generate protective biofilms that reduce $Na^+$ toxicity, and modulation of plant hormone levels (Ullah *et al.*, 2019). Verma *et al*. (2018) showed in their experiment that endophytic PGPR enhanced plant growth and development by improving root and shoot growth and restoring root geotropic response through root hair stimulation. The PGPR produced phytohormones like IAA in rice plants along with P solubilization. Furthermore, *Enterobacter* sp. reduced ethylene production in rice plants by enhancing ACC-deaminase synthesis under salinity stress (Sarkar *et al*., 2018b).

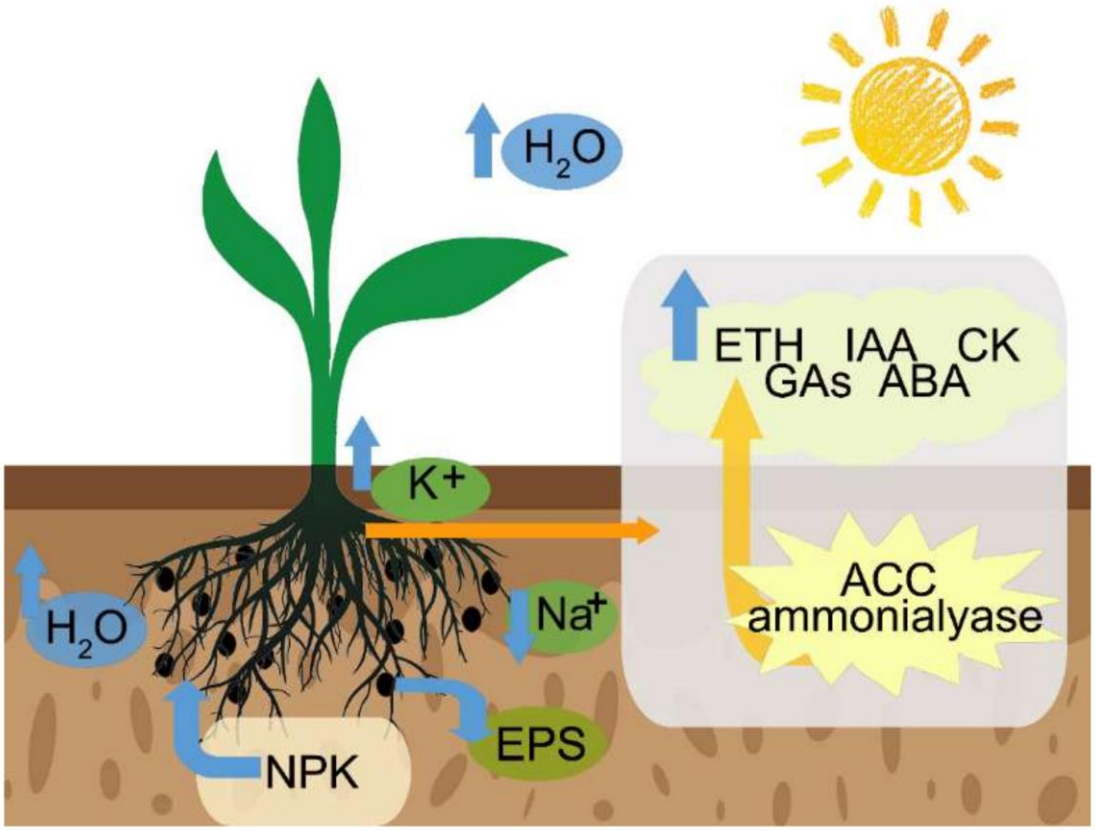


**Figure 4. The role of PGPRs in reducing salinity stress is explained. The PGPR is depicted by the black circles that encircle the roots. Under salt stress, plants reduce transpiration and water loss by increasing $K^+$ absorption and decreasing $Na^+$ absorption, alleviating osmotic stress and ionic stress; PGPRs promote plant growth by increasing nutrient absorption; in addition, PGPRs regulate hormone production (IAA, GAs, CK, and ABA) and ACC deaminase activity to alleviate salt stress** (Gao *et al.,* 2022)

## 2.9 Salt stress in plants is reduced by PGPR

### 2.9.1 Synthesis of ACC-deaminase

Plant growth-promoting rhizobacteria are known to accelerate plant development through two mechanisms: the synthesis of hormones like auxin, cytokinin, and gibberellin, and the suppression of ethylene by ACC deaminase. The ACC deaminase enzyme plays a crucial role in stimulating plant growth and increasing stress tolerance. It achieves this by converting the ethylene precursor ACC into ammonia and α-ketobutyrate (Ansari *et al*., 2019a). As a result, PGPR can effectively control the ethylene levels in plants. Research reports have indicated that the primary reasons for PGPR-mediated plant growth enhancement under salt stress are the production of ACC deaminase enzyme and a subsequent reduction in ethylene levels (Bhise *et al*., 2017).

**Table 2.** Salt stress tolerance by the inoculation of PGPR in different crops

| Crop | Bacteria | Activities | References |
|---|---|---|---|
| Maize (*Zea mays* L.) | *Bacillus* sp. | Siderophores production and phosphate solubilization | Ullah and Bano (2015) |
| Rice (*Oryza sativa* L.) | *B. amyloliquefaciens*-SN13 | Increased FW, DW, relative water content (RWC), decreased ROS and Pro | Chauhan *et al.* (2019) |
| Wheat (*Triticum aestivum* L.) | *Burkholderia* sp. MTCC 12259 | Enhanced synthesis of ACC deaminase | Sarkar *et al.* (2018a) |
| Wheat (*T. aestivum* L.) | *B. pumilus* strain FAB10 | Biofilm formation, enhanced EPS, auxin, ACC deaminase activities, and phosphate solubilization | Ansari *et al.* (2019b) |
| Peppers (*Capsicum annuum* L.) | 13 strains of *B. spp.* | Enhanced ACC deaminase activity and decreased ethylene formation | Wang *et al.* (2018) |
| Mung bean (*Vigna radiata* L.) | *Enterococcus* and *Pantoea* sp. | Increased plant growth and ACC deaminase activity | Panwar *et al.* (2016) |

### 2.9.2 Production of exopolysaccharides

Exopolysaccharides are primarily composed of monosaccharides, along with certain non-sugar substituents such as acetate, succinate, pyruvate, and phosphate. Free-moving rhizobacteria encounter the root surface, and upon attachment, induce biofilm formation (Kumar *et al*., 2020). Exopolysaccharides are produced by PGPR around the plant's root system, coating the root tip with biofilms. These biofilms provide resistance by binding to $Na^+$ ions, which enter the plant via salt water. As the EPS sequesters $Na^+$ ions, the concentration of these ions in the plant decreases, resulting in reduced salt accumulation. Therefore, EPS generated by PGPR enhances the plant's salinity tolerance.

### 2.9.3 Production of indole-3-acetic acid

Auxins, such as IAA, function as chemical messengers in bacterial and plant tissues. These compounds are produced through various metabolic and physiological processes, especially by numerous rhizospheric bacteria near the roots. Auxins increase the plant's biomass and surface area, facilitating enhanced water and nutrient absorption under both abiotic and biotic stress conditions (Patel *et al*., 2015). Approximately 80% of rhizospheric microbiomes produce and release phytohormones as secondary metabolites, altering native phytohormone synthesis by increasing cell membrane permeability for increased root exudate release (Bhise *et al*., 2017). Rhizospheric bacteria with auxin production capabilities can be used as bio-fertilizers and bio-enhancers. They augment root differentiation to enhance nutrient and water uptake by promoting auxiliary and adventitious root development. Kang *et al*. (2019) recently demonstrated that auxins produced by the salt tolerant *Leclercia adecarboxylata* strain MO1 significantly enhanced carbohydrate and Chl fluorescence ($F_v/F_m$) in tomatoes. The effects of various PGPR in mitigating salinity stress are demonstrated in Table 2.

### 2.9.4 Synthesis of siderophores

Iron, the fifth most abundant element in the Earth's crust, serves as a cofactor for over 140 enzymes including cytochrome and ribonucleotide reductase. It typically exists as insoluble ferric hydroxides and ferric oxides under conditions of sufficient oxygen, making it unavailable to plants and microorganisms. Siderophores are low-molecular-weight, high-affinity, water-soluble Fe-chelating compounds produced by microorganisms. These compounds scavenge Fe, preventing its utilization by phytopathogens. Parray *et al*. (2016) found that siderophore-producing *Pseudomonas* sp. GRP-3 increased Fe absorption in *Vigna radiata*, reducing chlorosis and enhancing Chl content compared to a control treatment.

### 2.9.5 Phosphate solubilization

Phosphorus is the second most important macronutrient after N, playing a crucial role in numerous metabolic processes affecting plant growth and health. Despite its abundant presence in the environment, a significant proportion of P is in insoluble forms. Soil microbes can render inorganic phosphate soluble in acidic soils, thus supplementing P under stressful environments. *B. aquimaris* was found to have a high phosphate content in salt-stressed wheat plants (Upadhyay and Singh, 2015). Phosphorus-solubilizing PGPR from genera such as *Cladosporium*, *Bradyrhizobium*, *Bacillus*, *Azotobacter*, *Pseudomonas*, and *Enterobacter* are prevalent and have been used to mitigate salinity stress in several crops.

## CHAPTER III

# MATERIALS AND METHODS

### 3.1 Experimental site

The experiment was conducted in the experimental shed house of the Department of Agronomy at Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location is positioned at 90°77' E longitude and at 23°77' N latitude with an altitude of 8.6 meters above sea level. This location is part of the AEZ 28 of the Madhupur tract or agroecological zone. The geographical location of the experiment site is displayed in Appendix I.

### 3.2 Climate and weather conditions

Located in a sub-tropical zone, this region experiences gusty winds in the Kharif season and sporadic rainfall in the Rabi season, accompanied by low temperatures. The experiment was conducted from January 2023 to May 2023. The monthly maximum and minimum temperatures, relative humidity, and total rainfall during this period were collected from the Bangladesh Meteorological Department in Dhaka, Bangladesh.

### 3.3 Plant materials

For this experiment, uniform, healthy, and fully viable rice seeds (*O. sativa* L. cv. BRRI dhan100, Bangabandhu dhan) were used. This is a Zn-enriched boro rice variety containing 25.7 mg $kg^{-1}$ Zn, released by the Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur, Bangladesh in 2020. As per (BRRI, 2020) the main characteristics of this rice variety include:

i. High yielding boro variety
ii. Life cycle: 148 days
iii. Upright, wide, and deep green flag leaf during the vegetative stage
iv. Plant height at maturity: 101 cm

v. Long, slightly slender, golden yellowish seeds

vi. 1000-seed weight: 16.7 g

vii. Average grain yield: 7 t $ha^{-1}$

viii. Protein and amylose content: 7.8% and 26.8%, respectively.

### 3.4 Seed collection and germination

Healthy seeds of rice were collected from BRRI, Joydebpur, Gazipur, Bangladesh for the experiment. The hard shells of rice seeds necessitate pre-sowing sprouting. After cleaning with running tap water, the seeds were soaked overnight in fresh water to expedite germination. They were then placed in a moist gunny bag until sprouting. Generally, seeds begin to sprout within 48 h and are ready for sowing in the seedbed within 72 h.

### 3.5 Rice seedling cultivation

Upon sprouting completion, the seeds were transferred to a seedbed for seedling growth. The seedbed was prepared through repeated plowing and laddering. No fertilizers were applied prior to seed sowing. The sprouted seeds were evenly spread across the seedbed for growing seedlings for transplantation. Regular weeding and irrigation ensured uniform seedling growth. Seedlings were grown for a month before being transplanted into the experimental pots.

### 3.6 Pot soil preparation and seedlings transplanting

The experiment utilized white plastic Wagner pots with dimensions of 10, 9, and 11 inches for height, bottom diameter, and top diameter, respectively. The pots were filled with 14 kg of thoroughly crushed, sun-dried soil to prepare for seedling transplantation. Wagner pots were chosen for their ease of irrigation management via a rubber stopper at the base of each pot. For seedling transplantation, the prescribed dose of fertilizers was mixed with the soil and puddled.

BRRI (2020) recommended fertilizer dose for BRRI dhan100 (Bangabandhu dhan) is presented below:

| Fertilizers | Dose (kg $ha^{-1}$) |
|---|---|
| Urea | 138 |
| TSP | 51 |
| MoP | 63 |
| Gypsum | 60 |
| $ZnSO_4$ | 4 |

The seedlings were carefully collected from the seedbed and transferred to a shed when they reached 42-day-old. Prior to uprooting, an ample amount of water was irrigated onto the seedbed to soften the soil and minimize root injuries and damages. In each Wagner pot, 5 hills with 2 seedlings were planted, maintaining a spacing of 15 cm × 15 cm during transplanting. However, only 2 hills in each pot were retained for the collection yield-contributing parameters.

### 3.7 Treatment combinations and their application to the plants

There were 12 treatments fixed for carrying out this experiment which are stated below:

i. Control
*ii.* *Bacillus subtilis* ($1 \times 10^9$ CFU $mL^{-1}$) (BS)
*iii.* *B. aryabhattai* (endophyte) ($3 \times 10^9$ CFU $mL^{-1}$) (BA endo)
*iv.* *B. aryabhattai* (epiphyte) ($3 \times 10^9$ CFU $mL^{-1}$) (BA epi)
*v.* $S_1$ (50 mM NaCl)
*vi.* $S_1$ + BS
*vii.* $S_1$ + BA (endo)
*viii.* $S_1$ + BA (epi)
*ix.* $S_2$ (100 mM NaCl)
*x.* $S_2$ + BS
*xi.* $S_2$+ BA (endo)
*xii.* $S_2$+ BA (epi)

This experiment involved the use of three PGPR culture solutions to mitigate the detrimental effects of salinity on rice. Two endophytic PGPR strains were used, namely, *B. subtilis* (CFU: $1 \times 10^9$ CFU $mL^{-1}$, Baciforte, Vijaya Agro Industries, Ahmednagar, India) and *B. aryabhattai* (CFU: $3 \times 10^9$ CFU $mL^{-1}$, sourced from the Department of Microbiology, University Dhaka), in addition to an epiphytic PGPR strain *B. aryabhattai* (CFU: $3 \times 10^9$ CFU $mL^{-1}$), also procured from the Department of Microbiology, University Dhaka. These bacterial cultures were applied thrice during the experiment: firstly, when seedlings were transplanted to the pot soils, secondly, five weeks post-transplantation during the vegetative stage, and lastly, during the panicle initiation stage, seven weeks after transplantation. The PGPR culture solutions were administered via two methods—seedling dipping and soil drenching—during transplantation. For seedling dipping, 100 seedlings were immersed in 20 mL of PGPR culture solutions for 60 minutes before transplantation. Additionally, all PGPR culture solutions were individually mixed with the puddled pot soil at a rate of 10 mL per pot.

Subsequently, two levels of salt stress, i.e., 50 and 100 mM of NaCl, were imposed on the plants via irrigation water, while the controls received only water. These salt stress treatments were administered twice, at seven-day intervals, starting five weeks after transplantation.

### 3.8 Experimental design and layout

A completely randomized design (CRD) with three replications was employed. The experiment was conducted using two sets of pots, one set being used for the collection of destructive data.

### 3.9 Intercultural operations

#### 3.9.1 Thinning and gap filling

Thinning and gap filling were performed as required to maintain a uniform plant density (5 hills per pot) in each pot.

### 3.9.2 Weeding

Regular checks were made for weeds, which were manually removed.

### 3.9.3 Irrigation

Irrigation was applied as needed, initially maintaining a 3 cm water level, increasing to 5-10 cm as the plant height increased. However, the frequency of irrigation was reduced during panicle initiation and all the water was drained out through the Wagner pot's rubber valves seven days prior to harvesting.

### 3.9.4 Plant protection measures

During the grain-filling stage, an attack of rice bug (*Leptocorisa acuta*) was observed. To counteract this, ACTARA (Thiamethoxam 25% WG) was sprayed at a concentration of 0.12 g $L^{-1}$ of water in the late afternoon, while observing appropriate precautions.

### 3.9.5 Harvesting and threshing

At 137 DAT, when 80% of the grains were mature and golden in color, they were harvested. The hills were then manually harvested using a sickle. Grains were separated from the straw using a pedal thresher, after which, the grains and straw were sun-dried separately to achieve a 12% moisture level.

## 3.10 Data collection

At harvest, yield and yield-related data were recorded. Upon completion of the treatment period, growth, physiological, biochemical, and phenotypic parameters were gathered.

### 3.10.1 Crop growth attributes

- Plant height
- Leaf area
- Shoot fresh weight per hill
- Shoot dry weight per hill

### 3.10.2 Physiological Attributes

- Relative water content
- Proline content
- Ion contents
- SPAD value of leaf
- Photosynthetic attributes, i.e., chlorophyll contents, $g_s$, $F_v/F_m$ ratio
- Indole-3-acetic acid (IAA) concentration

### 3.10.3 Oxidative stress indicators

- Lipid peroxidation
- $H_2O_2$ content
- Electrolyte leakage

### 3.10.4 Non-enzymatic antioxidants

### 3.10.5 Yield and yield contributing attributes

- Length of panicle
- Rachis number per panicle
- Filled grains per panicle
- Unfilled grains per panicle
- Total number of grains per panicle
- Number of effective tillers per hill
- Number of effective tillers per hill
- 1000-grain weight
- Total grain yield per hill
- Straw yield per hill

### 3.11 Procedures of crop growth attributes measurement

#### 3.11.1 Plant height

The plant height was measured from the soil surface to the tips of the leaves or panicle, was recorded at 62 days after transplanting (DAT), which marked the end of the treatment period. To calculate the plant height in cm, the heights of 5 plants from each distinct pot were measured and then averaged.

#### 3.11.2 Leaf area

Leaf area was measured using a length-width method at 62 DAT. The length of five randomly selected leaf blades was recorded using a measuring scale. The width (cm) of the leaf blades was taken from three distinct positions: base, middle, and top. Employing a constant (K) of 0.75, the LA was determined from the average length and width of five leaves and expressed in square centimeters ($cm^2$), as illustrated by the equation: Leaf area ($cm^2$) = K × Length (cm) × Width (cm)

#### 3.11.3 Plant fresh weight and dry weight

Upon conclusion of the treatment period, the FW of shoots from 5 hills per pot was recorded at 62 DAT. Plants were gently uprooted, rinsed with running tap water, and excess surface water removed before measurement. The average was then calculated and expressed as shoot FW per hill. After recording the FW, the samples were oven-dried for 72 h until they reached a consistent moisture content of 12%. The DW of 5 hills was then measured and averaged, expressing the result as shoot DW per hill.

### 3.12 Procedures for estimating physiological attributes

#### 3.12.1 Relative water content

The relative water content of leaves was calculated using the Barrs and Weatherly (1962) method, considering freshly measured leaf FW, DW, and turgid weight (TW). Freshly collected rice leaf blades were weighed, then left floating in water in Petri dishes overnight. The TW was recorded 12 h later after blotting the excess surface water from the leaf blades with a paper towel. The samples were then oven-dried for 48 h at 80 °C, and the DW was collected. The RWC was finally determined using the formula:
RWC (%) = (FW - DW) / (TW - DW) × 100

#### 3.12.2 Proline content

Bates *et al*. (1973) method was employed to determine the Pro concentration in leaf tissues. Fresh leaf samples weighing 0.5 g were homogenized with 3% sulfosalicylic acid using a pre-cooled mortar and pestle. The resultant solution was centrifuged at 11,500×g for 15 minutes to obtain the leaf extract. The supernatant was then mixed with equal volumes of acid ninhydrin and glacial acetic acid and incubated at 100 °C for an hthis. The reaction was halted by cooling the mixture in an ice bath for 15 minutes, after which 2 mL of toluene was added and vortexed for 20-30 seconds to separate the chromophore containing Pro. Following a 10-minute rest at room temperature, spectrophotometric absorbance was recorded at 520 nm using toluene as a blank. Laboratory-grade L-Pro served as the benchmark for determining Pro content within the linear ranges of 50-80 µM.

#### 3.12.3 Ion content

The $Na^+$ and $K^+$ contents of leaves were determined using a portable ion meter (Horiba, Tokyo, Japan). Sap extracted from freshly collected leaf samples (which had been rinsed with deionized water to remove residual dirt) was inserted into the ion meter's sensor, which had been calibrated using a standard solution.

### 3.12.4 SPAD value

Leaf soil and plant analysis development (SPAD) value was recorded using a LEAF (FT Green LLC, USA) SPAD meter from the top, middle, and bottom portions of 5 randomly selected leaf blades from each pot. The average value was then calculated and the total chlorophyll content was converted into SPAD units.

### 3.12.5 Chlorophyll content

After harvesting 0.25 g of fresh leaf samples from each treatment and chopping them into small pieces, they were subjected to a water bath with 10 mL of 100% ethanol to extract the chlorophyll pigments. The leaf samples were kept in the water bath until they turned white, indicating the complete extraction of Chl pigments. Subsequently, using a spectrophotometer, the colored chromophore was observed at wavelengths of 663, 645, and 470 nm. The concentrations of Chl *a*, Chl *b*, and Chl (*a*+*b*) pigments were determined following Arnon's (1949) method.

### 3.12.6 Stomatal conductance

Stomatal conductance was determined from fully expanded rice leaf surfaces using a leaf porometer (model SC-1, Decagon Devices, Inc., Pullman, WA, USA). Fully expanded leaves from individual plants across all treatments were labeled and readings were frequently taken in sunny conditions throughout the experiment. Data collection was avoided in wet conditions as $g_s$ depends on the amount of shade, sunlight exposure, leaf age, and plant placement. The device provides an accurate $g_s$ measurement in mmol $m^{-2}$ $s^{-1}$ within 30 seconds in auto mode.

### 3.12.7 Chlorophyll fluorescence

Chlorophyll fluorescence parameters provide insight into the activities of photosystem II (PSII) and photosystem I (PSI) under specific stress conditions. Leaf blades from randomly selected rice plants, from each treatment combination, were examined for chlorophyll fluorescence. A Hansatech Pocket PEA fluorometer was employed to

measure Chl fluorescence between 9 AM and 12 PM. The minimum fluorescence ($F_o$) was set after the device was adjusted using the supplied clips in a dark environment for 15 minutes. The maximum fluorescence ($F_m$) was obtained by exposing the leaf blades to a light pulse of 3000 μmol $m^{-2}$ $s^{-1}$ and recording the reading with the fluorometer. The maximum photochemical yield ($F_v/F_m$) of PSII in the dark-adapted state was calculated using the following equation: $F_v/F_m = (F_m-F_o)/F_m$.

### 3.12.8 Indole-3-acetic acid (IAA) concentration

The IAA concentrations in rice leaf samples from each treatment were measured following the procedure described by Gordon and Weber (1951). Initially, 0.5 g of leaves were collected and crushed using an ice-cooled mortar and pestle with 2 mL of 80% cold methanol to extract the IAA. The extract was then centrifuged at 5,000× g for 5 min, and 1 mL of the supernatant was pipetted into a 5 mL tube and mixed with 2 mL of Salkowski reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid). This mixture was kept in dark conditions for color formation after adding 2 drops of orthophosphoric acid. Two hthiss later, the optical density was measured spectrophotometrically at 530 nm and the final IAA concentration was determined using a standard IAA curve.

## 3.13 Procedures for estimating oxidative stress indicators

### 3.13.1 Malondialdehyde content

The malondialdehyde content, a lipid peroxidation biomarker, was measured following the method proposed by Heath and Packer in 1968, with minor modifications by Hasanuzzaman *et al.* (2022a). Briefly, 0.5 g of fresh leaf samples were homogenized with 3 mL of 5% trichloroacetic acid (TCA) before being centrifuged at 11,500×g for 15 minutes. After extracting the supernatants, 1 mL was mixed with 4 mL of thiobarbituric acid (TBA) reagent (20% TCA + 0.5% TBA) and then incubated in a water bath for 30 minutes. The spectrophotometric absorbance measurements at 532 nm and at 600 nm for non-specific absorbance were taken. The non-specific absorbance was later subtracted from the measurement at 532 nm for final calculations, using an extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$.

### 3.13.2 Hydrogen peroxide content

The $H_2O_2$ content was calculated following the method by Yu *et al.* (2003). In this method, 0.5g leaf samples were homogenized with 3 mL of 5% TCA, centrifuged at 11,500×g for 15 minutes to extract the supernatant. This supernatant was then incubated with potassium iodide and potassium-phosphate (K-P) buffer (pH 7.0) for 1 h at room temperature. Finally, the $H_2O_2$ concentration was determined spectrophotometrically at 390 nm, using an extinction coefficient of 0.28 $\mu M^{-1}$ $cm^{-1}$.

### 3.13.3 Electrolyte Leakage

Electrolyte leakage percentage (EL%) was measured according to the method of Dionisio-Sese and Tobita (1998). Freshly collected leaves (0.5g) were cut into small pieces and immersed in distilled water in a tube. The electrical conductivity ($EC_1$) was measured after heating them at 40 °C for 1 h, and the final conductivity ($EC_2$) was recorded after heating them at 121 °C. The EL was calculated using the following formula: $EL = (EC_1/EC_2) \times 100$.

### 3.14 Procedures for quantifying ascorbate and glutathione content

To determine the concentrations of non-enzymatic antioxidants AsA and GSH, 0.5 g of leaf samples were homogenized with 1 mM ethylenediaminetetraacetic acid (EDTA) in 5% meta-phosphoric acid (Nahar *et al.,* 2016). To determine the AsA pool, aliquots of total and reduced AsA were added to 0.1 M dithiothretitol (DTT) and distilled water, respectively, and neutralized with 0.5 M K-P buffer (pH 7.0). The concentrations of total and reduced AsA were measured spectrophotometrically at 265 nm, using a standard curve. The concentration of dehydroascorbate (DHA) was calculated by subtracting the concentration of reduced AsA from the total AsA.

To determine the GSH content, aliquots were oxidized with 5,5-dithio-bis (2-nitrobenzoic acid, DTNB) and neutralized with 0.5 M K-P buffer (pH 7.0) in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase (GR). The absorbance was measured at 412 nm. To neutralize the extract for oxidized glutathione (GSSG) measurement, 2-vinylpyridine and K-P buffer

were used. The GSH content was estimated by subtracting the GSSG from the total GSH, following the standard curves for GSH and GSSG (Hasanuzzaman *et al.*, 2018).

### 3.15 Procedures for assessing yield and yield parameters

#### 3.15.1 Effective tillers per hill

Tillers that produced panicles were considered effective. The number of effective tillers was calculated from two hills for each treatment. The average of these values represented the number of effective tillers per hill.

#### 3.15.2 Ineffective tillers per hill

Ineffective tillers, unlike effective ones, did not produce panicles. The number of ineffective tillers was calculated from two hills for each treatment, and the average number was reported per hill.

#### 3.15.3 Panicle length

Panicle length was measured from the base to the top of ten randomly chosen panicles from each treatment. The average length, expressed in centimeters, was then calculated.

#### 3.15.4 Rachis per panicle

The number of primary branches on ten panicles was counted for each treatment. The average number represented the number of rachis per panicle.

#### 3.15.5 Filled grains per panicle

The number of fully filled kernels from ten panicles of each treatment was counted and averaged to determine the number of filled grains per panicle.

### 3.15.6 Unfilled grains per panicle

Unfilled grains were defined as partially filled or unfilled kernels. The number of unfilled grains per panicle was determined in the same manner as filled grains, with the average number of unfilled grains from ten randomly selected panicles calculated for each treatment.

### 3.15.7 Total number of grains per panicle

The total number of grains per panicle was calculated by adding the number of filled and unfilled grains per panicle.

### 3.15.8 1000-grain weight

One thousand grains from each treatment were counted using a seed counter and weighed. The result was reported in grams.

### 3.15.9 Grain yield per hill

After threshing and winnowing, the grains were sun-dried to a moisture content of 12%, and their weight was recorded. The average weight represented the grain yield per hill.

### 3.15.10 Straw yield per hill

Once the grains were separated and dried to a constant weight, the weight of the straw from two hills of each treatment was measured. The average weight was expressed in grams per hill.

## 3.16 Phenotypic observations

The phenotypic characteristics of each treatment were evaluated, and photos were taken using a digital camera.

### 3.17 Statistical analysis

Data from various parameters were subjected to an analysis of variance (ANOVA) using CoStat v.6.400 (2008). Mean separations were compared using the Tukey's HSD test at a 5% level of significance.

# CHAPTER IV

# RESULTS AND DISCUSSION

## 4.1 PGPR and their effect on the growth attributes of rice under salt stress

### 4.1.1 Plant height

Plant height was reduced by 14% and 17% under 50 mM and 100 mM NaCl stress, respectively, when compared to the control (without NaCl). However, the application of endophytic (*B. subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) PGPR counteracted the adverse effects of salt stress under both levels of salt stress conditions. *B. subtilis* demonstrated superior performance, enhancing the height of rice plants more significantly under both salinity conditions than other strains. Specifically, *B. subtilis* enhanced rice plant height by 7% and 8% under 50 mM and 100 mM NaCl stress conditions, respectively (Figure 5A).

### 4.1.2 Biomass

Rice plants exposed to two different levels of salinity showed a notable reduction in both FW and DW relative to the controls (Figure 5B, 5C). Under 50 mM and 100 mM NaCl stress, FW decreased by 58 and 63%, respectively, while DW declined by 35% and 43%, respectively. The application of PGPR showed beneficial effects primarily at the lower NaCl dose, enhancing FW, but demonstrated a different trend for DW. Notably, *B. subtilis* application resulted in the highest increase in FW (51%) under 50 mM NaCl stress. However, under 100 mM NaCl stress, the PGPR did not produce significant benefits. In terms of DW, both *B. subtilis* and epiphytic *B. aryabhattai* outperformed other PGPRs, increasing DW by 25% under 50 mM NaCl stress.

### 4.1.3 Leaf area

A reduction in LA was observed following exposure to two different salinity levels. Leaf area was reduced by 32 and 35% under 50 mM and 100 mM NaCl stress, respectively, compared to controls (Figure 5D). Nevertheless, the application of PGPR mitigated this reduction, increasing the LA under both 50 mM and 100 mM NaCl stress. Among them *B. subtilis* was the most effective, increasing the LA of rice plants by 22 and 16% under 50 mM and 100 mM NaCl stress conditions, respectively. The enhancements by both endophytic and epiphytic *B. aryabhattai* were statistically similar under 50 mM and 100 mM NaCl stress conditions.

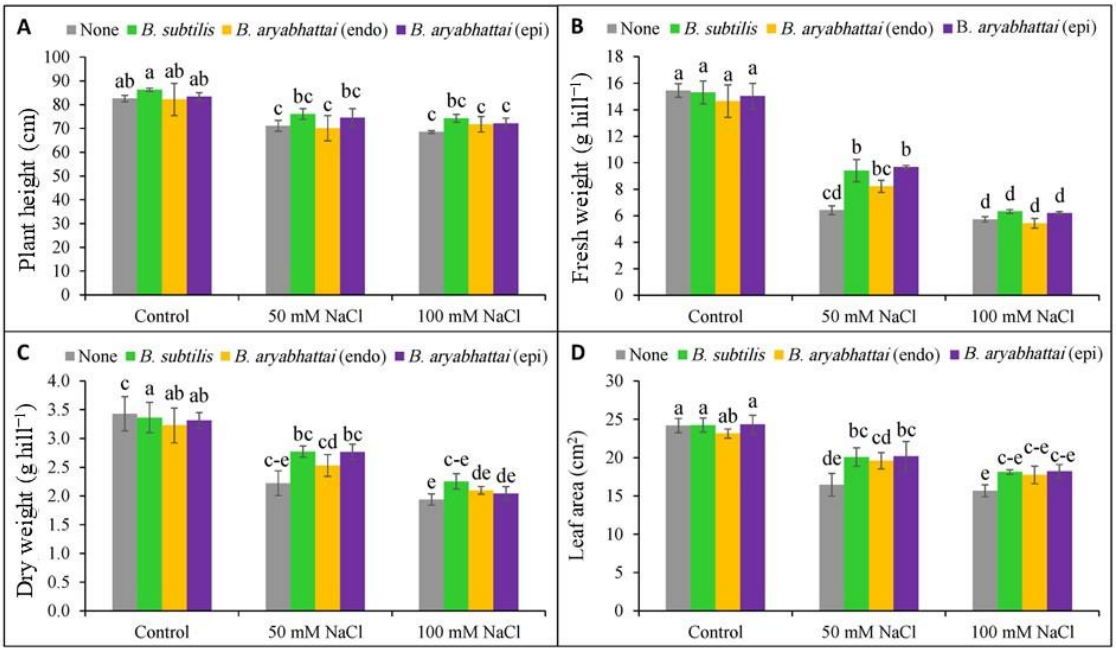


**Figure 5. Variations in plant height (A), fresh weight (B), dry weight (C), and leaf area (D) of rice (*Oryza sativa* L. cv BRRI dhan100) plants under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

The initial response of plants to salinity stress involves osmotic shock and ionic imbalance. The most immediate reaction, osmotic stress, disrupts water uptake and leads to the closure of stomatal openings. This stress response is among the factors that restrict plant growth, development, cell division, and enlargement (Hasanuzzaman *et al.*, 2021a). High salt concentrations around plant roots produce a direct osmotic effect, lowering their water absorption ability. In this study, salinity stress severely impeded the growth attributes of rice plants, including plant height, FW, DW, and LA corroborating previous research. However, recent advancements in microbiology have demonstrated that the application of beneficial bacteria can enhance plants' salinity tolerance.

One vital growth-promoting hormone under stress is IAA, whose production is stimulated by PGPR (Gupta *et al.,* 2022). Moreover, microorganisms effectively convert inaccessible minerals into forms readily absorbed by plants, promoting plant growth. In this study, the application of PGPR improved the height, FW, DW, and LA of rice plants, attributable to the PGPR-induced synthesis of IAA and siderophores, which facilitated plant growth under stressful conditions.

The findings align with those of Shultana *et al.* (2021), who investigated the proficiency of various *Bacillus* strains in solubilizing phosphorus (P) and potassium (K). Furthermore, the production of siderophore by *Bacillus* sp., converting unavailable Fe forms into accessible ones, increased root length, plant height, and stem diameter of chili under salt stress (Ansari *et al.*, 2019a).

## 4.2 Changes in photosynthetic attributes of salt-treated rice with PGPR application

### 4.2.1 Chlorophyll contents

Chlorophyll *a* and Chl *b* contents in rice leaves decreased significantly under salinity stress compared to the control. This decline eventually led to the reduction of total Chl (*a*+*b*) content. However, salinity-stressed plants treated with PGPRs showed significantly increased amounts of Chl *a*, Chl *b*, and Chl (*a*+*b*) content compared to stressed plants (Figure 6A, 6B, 6C). Plants treated with 50 mM NaCl showed a 17, 42,

and 30% reduction in Chl *a*, Chl *b*, and Chl (*a*+*b*), respectively, compared to the control. Higher stress (100 mM NaCl) increased this reduction to 33, 63, and 48% for Chl *a*, Chl *b*, and Chl (*a*+*b*), respectively. The adverse effects of salt stress were mitigated with the application of PGPRs in all cases. Although endophytic *B. aryahattai* had the lowest effect under both stress levels for all Chl pigments, endophytic *B. subtilis* and epiphytic *B. aryabhattai* proved to be more beneficial.

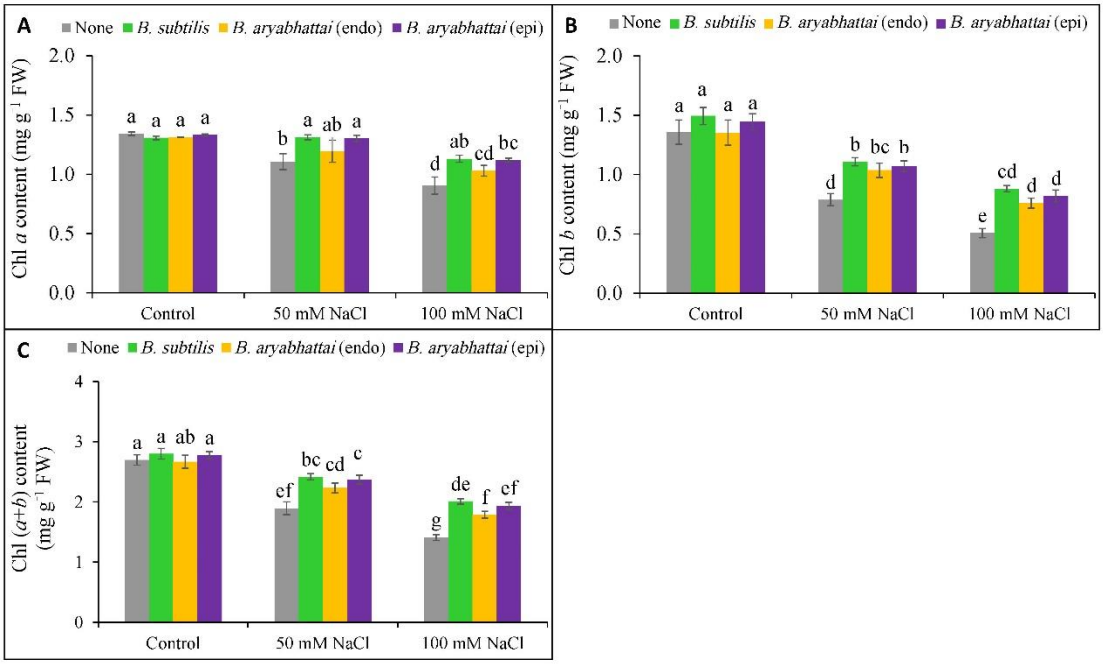


**Figure 6. Variations in Chl *a* content (A), Chl *b* content (B) and Chl (*a*+*b*) content (C) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Salinity induces physiological drought in plants, leading to stomatal closure, delayed photosynthetic $CO_2$ uptake, and ultimately a decrease in Chl content and photosynthesis

efficiency. According to Puthiyottil and Akkara (2021), salt-induced stress prompts the photosynthetic pigments (Chl *a*, Chl *b*, Car) to contract, triggering an accelerated generation of ROS due to the activation of the chlorophyllase enzyme. Furthermore, magnesium (Mg), a vital component of Chl *a* and Chl *b*, when depleted, negatively impacts the rate of photosynthesis (Tränkner *et al.*, 2018). This study observed a significant decrease in the photosynthetic pigment contents of rice plants due to salt stress, which increased after *Bacillus* strains application. The microbial solubilization of Fe and Mg, combined with the stress-induced synthesis of siderophores by them, may have contributed to the regeneration of the photosynthetic pigments (Ansari *et al.*, 2019a; Ferreira *et al.*, 2019).

### 4.2.2 SPAD Value

Under both 50 and 100 mM NaCl stress, rice demonstrated a decrease in SPAD value across all treatments relative to the control. Interestingly, the value was reverted with the intensification of salinity levels, eventually leading to the highest reduction (14%) in SPAD value under the highest salinity level (Figure 7A). In contrast, the application of endophytic *B. subtilis* and *B. aryabhattai* and epiphytic *B. aryabhattai* increased the SPAD value by 9, 6, and 11%, respectively, under 100 mM NaCl stress. The epiphytic *B. aryabhattai* outperformed the others.

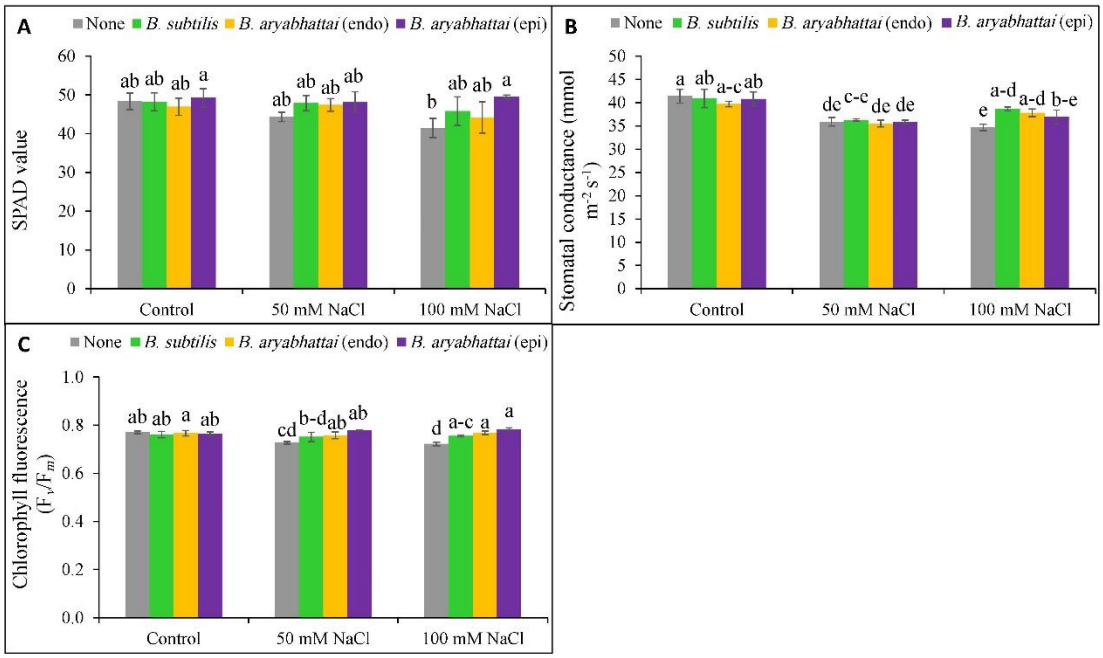


**Figure 7. Variations in SPAD value (A), stomatal conductance (B) and chlorophyll fluoroscence (C) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

The effectiveness of all the *Bacillus* strains was non-significant under 50 mM NaCl-induced salinity stress in improving the SPAD value of rice plants. The increase was only 8, 7, and 8% with the application of *B. subtilis*, *B. aryabhattai*, and *B. aryabhattai*, respectively, under mild stress conditions.

### 4.2.3 Stomatal conductance

Under all treatments, rice plants showed a reduction in stomatal conductance ($g_s$) after the application of 50 and 100 mM NaCl-induced salt stress, compared to the control (7B).

### 4.2.4 Chlorophyll fluorescence

A reduction in Chl fluorescence ($F_v/F_m$ ratio) was observed when subjected to salt stress induced by 50 and 100 mM NaCl across all treatments relative to the control. Though this reduction increased proportionately with the severity of the salinity level, the most significant reduction (7%) in $F_v/F_m$ was observed under 100 mM NaCl-induced salinity stress (Figure 7C). However, with the application of endophytic *B. subtilis* and *B. aryabhattai* and epiphytic *B. aryabhattai*, it increased by 5, 6, and 8% respectively under higher salinity stress, with epiphytic *B. aryabhattai* performing the best.

Similarly, the performance of all the PGPRs improved under 50 mM NaCl-induced salinity stress, enhancing the $F_v/F_m$ ratio of rice plants. The $F_v/F_m$ ratio increased by only 3, 4, and 7% with the application of *B. subtilis*, *B. aryabhattai*, and B. aryabhattai respectively, under 50 mM NaCl stress conditions, where *B. aryabhattai* performed the best.

Salinity causes physiological drought in plants, leading to stomatal closure, delayed photosynthetic $CO_2$ uptake, and a decrease in Chl content and other photosynthetic attributes, such as a reduction in SPAD value, $g_s$, and $F_v/F_m$. Puthiyottil and Akkara (2021) found that salt-induced stress caused photosynthetic pigments (Chl *a*, Chl *b*, Car) to contract and accelerated ROS generation due to the activation of the chlorophyllase enzyme. Furthermore, the decrease in Mg, a key component of Chl *a* and Chl *b*, negatively impacts photosynthesis rates, causing chlorosis, necrosis, and leaf senescence (Tränkner *et al.*, 2018). In this study, a reduction in SPAD value under salt stress was noted, indicative of Chl pigment degradation. This aligns with findings from Hasanuzzaman *et al.* (2021b), who observed a reduction in SPAD value in jute plants under increasing levels of salinity. However, PGPR application improved the SPAD value in this experiment. Similar reductions in $g_s$ were observed in this study and were subsequently restored after the PGPR application. These findings echo results from previous experiments (Senguttuvel *et al.*, 2014; Abd El-Mageed *et al.*, 2022). The reduction is likely due to the disruption of water balance in plant cells due to increasing salt stress. Plants close their stomata to prevent excessive transpiration water loss and

consequently reduce $CO_2$ uptake required for photosynthesis. As PGPR application improved the $g_s$ of rice plants, it may be due to improved root growth, nutrient and water uptake, leading to better stomatal movement and improved photosynthetic gaseous exchange. This result aligns with findings from Zheng *et al*. (2019) and Abd El-Mageed *et al*. (2022).

Moreover, PGPR application can positively impact the parameters of chlorophyll fluorescence in rice plants under salt stress. In this study, salt-induced toxicity reduced the ratio of maximum quantum yield ($F_v/F_m$) of Chl fluorescence, which aligns with findings from Tsai *et al*. (2019). However, treatment with PGPR strains increased photosynthetic efficiency, demonstrated by increased $F_v/F_m$. Results from Abd El-Mageed *et al*. (2022) showed similar outcomes. This might be due to the restoration of photosynthetic pigments by siderophore production by the PGPR that reintroduced Fe and Mg molecules.

### 4.3 Effect of PGPR on the physiological attributes of rice under salt stress

#### 4.3.1 Osmotic adjustment and changes in relative water content

The RWC was reduced by 16% and 26% with the salt treatment of 50 and 100 mM NaCl, respectively, compared to the control. However, a significant increase in RWC was seen with the application of endophytic *B. subtilis* by 14 and 19% in lower and higher doses of salt-stressed plants compared to salt only (Figure 8A). The epiphytic *B. aryabhttai* and endophytic *B. aryabhattai* were unable to mitigate the effects of 50 mM salt stress, but they worked better for 100 mM salt stress, improving the RWC of rice leaves by 13 and 20%, respectively.

Compared to the control, Pro content significantly increased in rice plants when exposed to increasing levels of salinity stress. Specifically, plants treated with 50 and 100 mM NaCl stress, showed a significant increment of 136 and 327% Pro, respectively than the controls (Figure 8B). The application of PGPR improved this condition by reducing the excessively generated Pro content under 50 mM NaCl stress (25% reduction by *B. subtilis* than the salt stress alone). There was very little change observed under higher salinity levels as it was only 16 and 10% with *B. subtilis* and epiphytic *B.*

*aryabhattai,* respectively, whereas, no change was found with endophytic *B. aryabhattai* under 100 mM NaCl stress (Figure 8B).

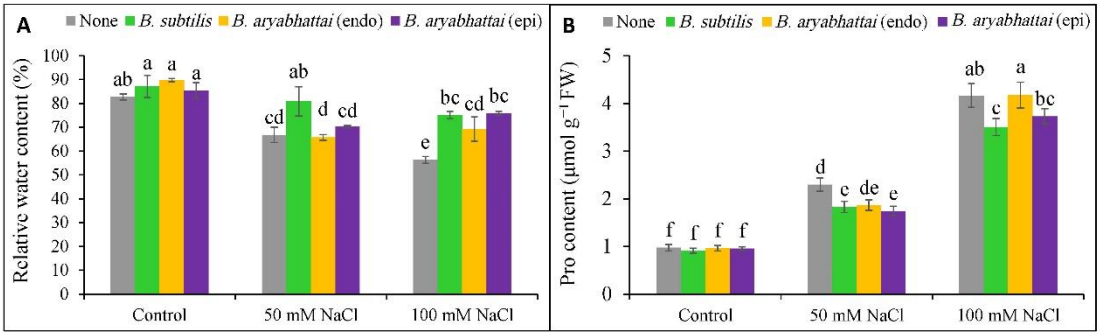


**Figure 8. Variations in relative water content (A) and Proline (Pro) content (B) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Stomata serve a critical role in maintaining osmotic balance under salt stress. A decline in $K^+$ content impairs stomatal regulation in plants exposed to saline environments, ultimately disrupting the osmotic balance of plant cells (Barragán *et al.*, 2012). To cope, plants produce excess osmolytes (such as Pro) to mitigate osmotic stress (Munns, 2011). In this study, salinity-induced osmotic stress was evident through increased osmotic potential, Pro accumulation, and diminished leaf RWC. However, treatment with PGPR enhanced the RWC in salt-stressed rice plants and lessened Pro build-up. This is likely due to the EPS produced by PGPR, which forms a protective biofilm within bacterial cells. These findings align with the study of Ji *et al.* (2022), which highlighted how wheat seedlings inoculated with PGPR under salt stress could stave off osmotic stress by regulating Pro and soluble sugar accumulation.

### 4.3.2 Effect of PGPRs in maintaining ion homeostasis in rice plants under salt stress

The application of 50 and 100 mM NaCl stress disrupted the ion homeostasis in rice plants, as evidenced by increased $Na^+$ accumulation and the $Na^+/K^+$ ratio, as well as decreased $K^+$ accumulation, compared to control plants (Figure 9A, 9B, 9C). Rice leaves amassed almost 32- and 39-fold higher $Na^+$ (Figure 9A) under 50 and 100 mM NaCl stress, respectively, than untreated plants. Conversely, $K^+$ uptake decreased by 15 and 22% (Figure 9B), leading to a 40- and 53-fold increase in the $Na^+/K^+$ ratio due to elevated $Na^+$ uptake.

Nevertheless, applying beneficial plant microbes reversed this imbalance by preserving ion homeostasis, reducing $Na^+$ accumulation, and enhancing $K^+$ uptake through rice plant roots. As a result, the greatest reduction (78 and 81% under 50 and 100 mM NaCl stress, respectively) in $Na^+$ was noted with *B. subtilis*, leading to a 30 and 67% increase in $K^+$ uptake (Figure 9B). This restored the $Na^+/K^+$ ratio by nearly 83 and 89% (Figure 9C) under 50 and 100 mM NaCl stress, respectively.

Although endophytic *B. aryabhattai* demonstrated superior performance under mild NaCl stress by decreasing $Na^+$ content by 73%, it was less effective under higher salinity conditions, reducing $Na^+$ only by 43% compared to untreated salt-stressed plants. However, under 100 mM NaCl stress, $K^+$ uptake was higher (almost 33%) than under 50 mM NaCl stress (only 9%) with this bacteria (Figure 9B). Consequently, the $Na^+/K^+$ ratio was reduced (approximately 75%) under lower level of salt stress with the application of endophytic *B. aryabhattai*.

In contrast, with epiphytic *B. aryabhattai*, the outcome was reversed, as the reduction was greater (64%) under high salinity levels than under lower ones (59%). However, $K^+$ accumulation and the restoration of the $Na^+/K^+$ ratio was nearly identical in both cases (Figure 9B, 9C). Therefore, among the three PGPRs, *B. subtilis* was identified as the most effective microbe.

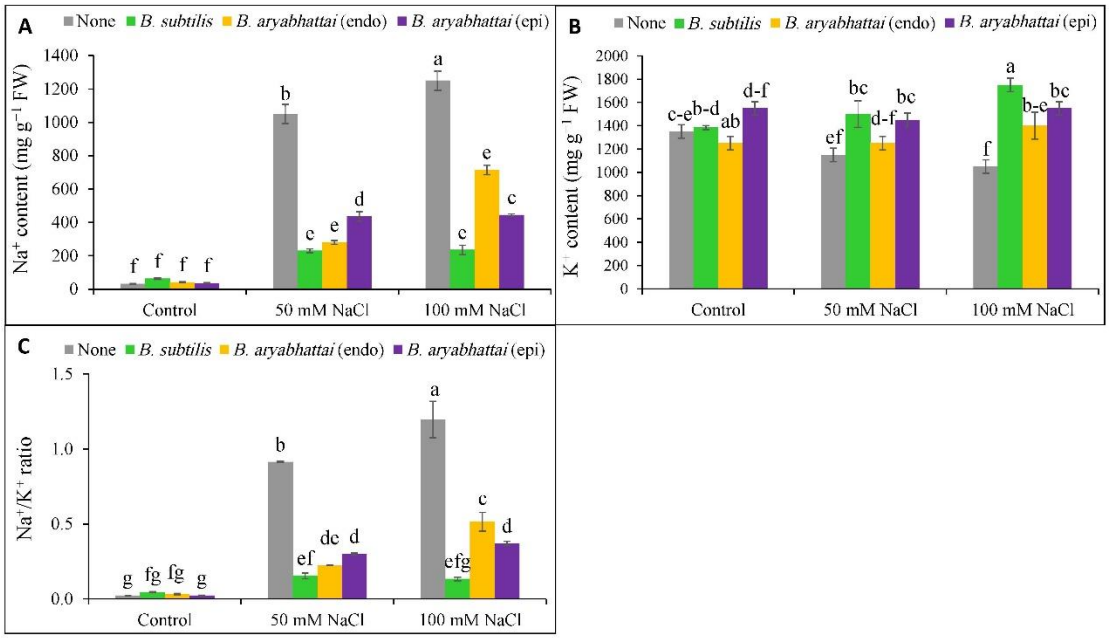


**Figure 9. Variations in $Na^+$ content (A), $K^+$ content (B) and $Na^+/K^+$ ratio (C) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Salinity primarily impacts plants by causing osmotic stress and ionic toxicity. The primary response to salt stress is osmotic stress, resulting from excessive uptake of $Na^+$ which subsequently leads to ionic toxicity. Therefore, under saline conditions, ionic homeostasis and critical cellular functions, such as nutrient absorption and translocation, are compromised by salt-induced ionic toxicity. This study examining rice under salinity stress found that salt stress disrupted ion homeostasis by increasing $Na^+$ accumulation, reducing $K^+$ absorption, and raising the $Na^+/K^+$ ratio. Similar ionic imbalances caused by salinity in rice have been reported in earlier studies (Chen *et al*., 2022; Khan *et al*., 2022; Rahman *et al*., 2023). However, the application of PGPR enhanced ion homeostasis in salt-treated rice plants by decreasing $Na^+$ accumulation and increasing $K^+$ absorption through the roots. This improvement can be attributed to

the release of EPS by PGPR that obstructs $Na^+$ deposition on root surfaces under salinity, thus promoting systemic tolerance to salt stress (Shultana *et al.*, 2020).

### 4.3.3 Effect of PGPRs on indole-3-acetic acid content in rice under salt stress

In comparison to the control, the concentration of IAA significantly decreased in rice plants exposed to increasing levels of salinity stress. Specifically, plants subjected to 50 and 100 mM NaCl stress demonstrated a significant IAA reduction by nearly 15 and 32%, respectively, compared to the controls (Figure 10). However, the application of PGPRs ameliorated this condition by boosting IAA concentrations under both mild and severe salinity conditions. Notably, among the three PGPRs, epiphytic *B. aryabhattai* was the most effective under both salinity levels, increasing IAA concentrations in rice plants by approximately 49 and 92%, respectively, compared to stressed plants.

However, changes were relatively minor under higher salinity conditions, showing only a 27 and 21% increase with *B. subtilis* under 50 and 100 mM NaCl, respectively. Conversely, a significant increase was observed with endophytic *B. aryabhattai* under 100 mM NaCl stress (Figure 10).

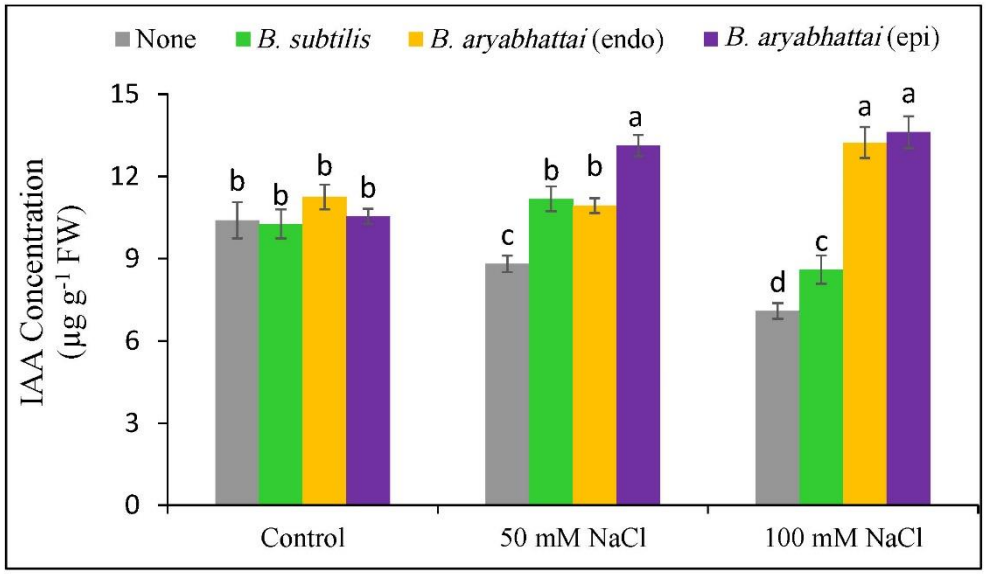


**Figure 10. Variations in indole-3-acetic acid (IAA) concentration of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant**

**growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Indole-3-acetic acid, also known as auxin, is produced by several rhizospheric bacteria in the vicinity of plant roots and induces a variety of physiological changes, such as enhancing root length, surface area, and nutrient uptake under stressful environments. Auxins influence Chl pigment development, seed germination, cell division and differentiation, and ultimately photosynthesis. Salinity stress reduces IAA synthesis, thereby impeding plant growth and development and interfering with phytohormone production. In this current study, IAA synthesis decreases with increasing levels of salinity. However, the application of PGPR mitigates this condition by promoting IAA production. This outcome aligns with the findings of Habib *et al*. (2016), who demonstrated that *Bacillus* sp. enhanced IAA production under saline conditions, thereby inducing salt tolerance. Another study with mungbean also indicated similar results, with IAA production mitigating salt stress following the inoculation of *Bacillus* sp. (Islam *et al.*, 2016). Shultana *et al*. (2020) reported comparable results with *B. aryabhattai* and *B. subtilis*, showing increased levels of IAA production under saline stress, supporting these experimental findings.

## 4.4 The beneficial effect of PGPRs on the biochemical attributes of rice under salt stress

### 4.4.1 Oxidative stress indicators

Salt stress prompts the production of an excessive amount of ROS, disrupting cellular redox balance and ultimately causing significant oxidative damage to plants. Lipid peroxidation, a marker of oxidative stress, is instigated by such stress, causing lipid membrane damage. Malondialdehyde content is a key indicator of lipid peroxidation under stressful conditions, and it's quantified to gauge the level of oxidative stress or the degree of lipid peroxidation.

A significant rise in MDA content was observed with increasing salinity levels, leading to membrane damage in rice plants. In this experiment, the highest lipid peroxidation, almost 58%, was noted under 100 mM of NaCl stress compared to the controls. However, PGPR treatment significantly reduced the MDA content in both stress conditions. Under 50 and 100 mM NaCl stress, nearly 31 and 29% MDA content was diminished due to *B. subtilis* application compared to salt stress alone plants, respectively (Figure 11A). The effect of endophytic PGPR, *B. aryabhattai*, was not as profound, with reductions only amounting to 17 and 18% for 50 and 100 mM NaCl stress, respectively. However, epiphytic PGPR *B. aryabhattai* resulted in a consistent level (25%) of reduction in MDA content at both levels of salt stress.

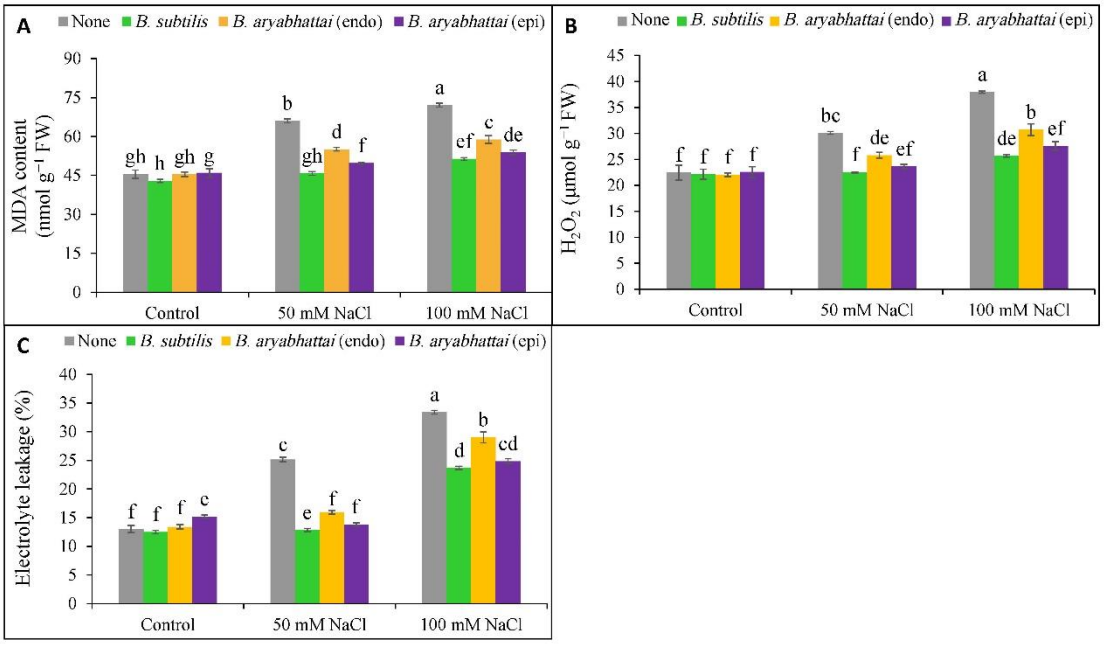


**Figure 11. Variations in MDA content (A), $H_2O_2$ content (B) and electrolyte leakage (%) (C) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

An increase in salinity doses corresponded with a rise in $H_2O_2$ levels, leading to membrane damage in rice plants. Under 100 mM of NaCl stress, $H_2O_2$ levels rose by approximately 69% compared to the controls. However, treatment with PGPRs notably mitigated this effect. Under 50 and 100 mM NaCl stress, the application of *B. subtilis* reduced $H_2O_2$ levels by roughly 25 and 32%, respectively, compared to the untreated plants (Figure 11B). The impact of the endophytic *Bacillus aryabhattai*, was less pronounced, leading to a reduction of only 14 and 19% under the same conditions. However, the application of epiphytic *B. aryabhattai* led to a 21 and 27% decrease in $H_2O_2$ content at both stress levels.

Likewise, EL levels in rice plants also increased under salinity stress, mirroring the trend observed with MDA and $H_2O_2$ contents. The highest EL percentage was around 20% under 100 mM NaCl stress, almost double that under 50 mM of NaCl and compared to controls. PGPR treatment mitigated this increase; under 50 and 100 mM NaCl stress, *B. subtilis* application led to approximately 12 and 10% decreases in EL levels (Figure 11C). The application of endophytic *B. aryabhattai* resulted in lesser reductions of 9 and 4%, while epiphytic *B. aryabhattai* led to an 11 and 9% reduction.

The present study reveals that salinity-induced oxidative stress in rice plants, as demonstrated by increased ROS generation, MDA content, and EL levels. However, PGPR addition inhibited ROS production and lipid peroxidation in salt-treated rice plants by reducing MDA content and EL levels. This aligns with prior studies indicating the potential of *B. subtilis* and *B. aryabhattai* as soil microbes that lower MDA and $H_2O_2$ contents in plants by detoxifying ROS and boosting antioxidant defense.

### 4.4.2 Effect of PGPRs on antioxidant defense in salt-stressed rice plants

#### 4.4.2.1 PGPRs and the AsA-GSH pool under salt stress

Salt stress levels inversely affected AsA content. However, the application of PGPRs offsets this stress by increasing AsA content. Under 100 mM NaCl stress, AsA content was reduced by 53%, while a 29% reduction was observed under 50 mM NaCl stress (Figure 12A). *B. subtilis* was particularly effective, increasing AsA levels by 15 and

27% under 50 and 100 mM NaCl stress respectively. However, endophytic *B. aryabhattai* only led to a 6% reduction under 50 mM NaCl stress and had no effect under 100 mM NaCl stress. The application of epiphytic *B. aryabhattai* led to a 16% reduction under mild salt stress, with no noticeable effect under higher stress levels.

Saline conditions caused a remarkable increase in DHA content compared to controls. In contrast, PGPR treatment reduced DHA levels in salt-stressed rice plants. The highest DHA content (89%) was observed under 100 mM NaCl stress, approximately 1.5 times higher than under 50 mM NaCl stress (Figure 12B). However, PGPRs ameliorated this effect; *B. subtilis* and epiphytic *B. aryabhattai* respectively reduced DHA content by 16 and 12% at 50 and 100 mM NaCl stress, while endophytic *B. aryabhattai* showed a similar trend.

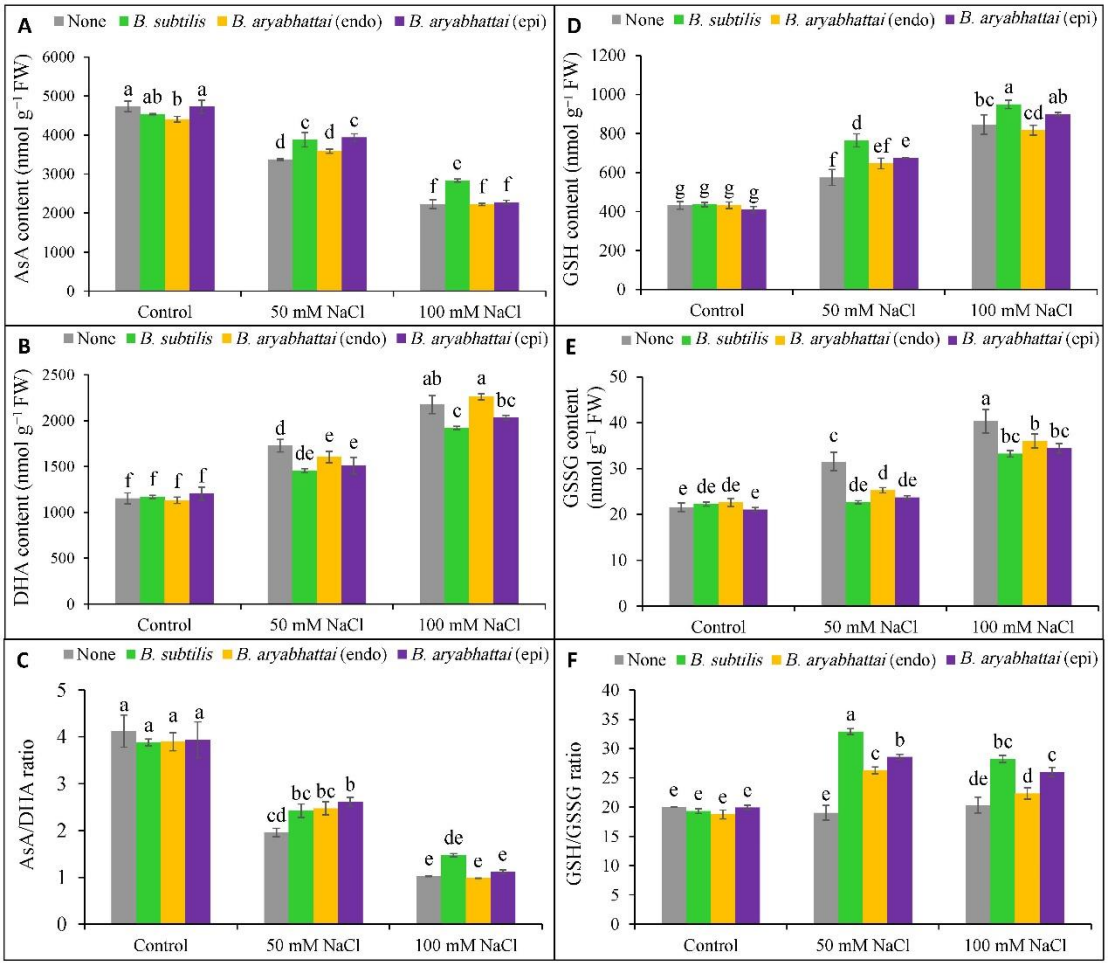


**Figure 12. Variations in AsA content (A), DHA content (B), AsA/DHA ratio (C), GSH content (D), GSSG content (E) and GSH/GSSG ratio (F) of rice (*Oryza sativa* L. cv BRRI dhan100) leaves under varying levels of salt**

**stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Under 50 and 100 mM NaCl stress, the AsA/DHA ratio decreased by 53 and 75%, respectively, due to salt stress-induced reduction in AsA content and increase in DHA contents. However, applying endophytic PGPRs *B. subtilis* and *B. aryabhattai* increased the ratio by 24 and 26%, respectively, under 50 mM NaCl stress, compared to plants only subjected to salt stress. Furthermore, epiphytic PGPR *B. aryabhattai* increased the ratio by 34% (Figure 12C). However, with the exception of *B. subtilis*, other PGPRs could not revert the increased AsA/DHA ratio under higher salinity levels.

Compared to the control, GSH content increased by 33 and 94% under 50 and 100 mM NaCl stress, respectively (12D). The application of PGPRs further enhanced the GSH content under both salt stress conditions. The most substantial increase was found with *B. subtilis* application: a 25 and 12% increase at mild and severe saline conditions compared to the salt-stressed plants only. The effect of other PGPRs was not significant at higher saline doses.

The level of oxidized glutathione (GSSG) content significantly increased by 46 and 87% under 50 and 100 mM NaCl stress, respectively, compared to the controls (12E). However, PGPRs treatment reduced the GSSG levels in salt-stressed plants. The most significant GSSG content reduction, about 28 and 18%, was observed at 50 mM NaCl stress with *B. subtilis* application. Furthermore, epiphytic PGPR *B. aryabhattai* and endophytic PGPR *B. aryabhattai* reduced the GSSG content by 25% compared to the salt-stressed plants only. However, the effect of PGPRs in reducing GSSG content was not significant at higher salt stress levels.

The severity of the stress substantially decreased the GSH/GSSG ratio compared to the control. However, PGPR treatment recovered the GSH/GSSG ratio in salt-stressed rice

plants. The most significant improvement in the ratio, 39 and 73%, was observed at 50 and 100 mM NaCl stress with the endophytic *B. subtilis* application(12F). The epiphytic PGPR, *B. aryabhattai*, performed better in increasing the GSH/GSSG ratio under both salt-stressed conditions, improving by nearly 50 and 28% under 50 and 100 mM NaCl stress, respectively. However, the endophytic PGPR, *B. aryabhattai*, was not as effective in reverting the GSH/GSSG ratio at both salinity stress levels, increasing the ratio by nearly 38% at 50 mM NaCl stress but showing a 3-fold lesser reduction under higher salinity stress. Therefore, among the three PGPRs, *B. subtilis* was most effective in restoring the AsA-GSH pool of salt-induced rice plants.

Plants activate the ROS-scavenging system, comprising non-enzymatic and enzymatic antioxidants, to protect themselves from the oxidative injury caused by ROS under salt stress. Non-enzymatic antioxidants AsA and GSH are highly effective in preventing ROS production and regulating homeostasis, thereby safeguarding plant cells from oxidative damage (Mahmud *et al.*, 2020). Ascorbate (AsA) is involved in the production of phytohormones that inhibit ROS production by providing electrons to APX in stressful situations. Conversely, being a low-molecular thiol tripeptide, GSH, along with GPX, breaks down $H_2O_2$ into $H_2O$ and $O_2$ (Hasanuzzaman *et al.*, 2019). Therefore, AsA and GSH, either individually or together, are crucial for stress detection and response.

In this study, salt stress led to an increase in DHA (the oxidized form of AsA), which decreased AsA and the AsA/DHA ratio. Consequently, salinity enhanced the buildup of ROS in the rice plants, aligning with the earlier findings of El-Esawi *et al*., (2018) and Hasanuzzaman *et al.* (2021).

This study also revealed that rice plants under salt-induced oxidative stress produced more GSH and had a lower GSH/GSSG ratio. The overproduction of ROS caused GSH to be oxidized, resulting in more GSSG production and a decreased GSH/GSSG ratio in salt-treated rice seedlings. Similar conclusions were drawn from other studies (Zhu *et al*., 2020; Soliman *et al*., 2020), which found that salinity decreased the GSH/GSSG ratio in plants while increasing the production of GSSG. However, adding PGPR to salt-treated rice seedlings improved the GSH/GSSG ratio by boosting GSH production and lowering GSSG levels. An increase in the AsA/DHA and GSH/GSSG ratios following *B. subtilis* inoculation suggests that ROS detoxification under salt stress is

more effective as AsA and GSH levels were elevated and DHA and GSSG contents were decreased. This finding is consistent with previous research showing that PGPR inoculation increased AsA and GSH synthesis in wheat (Maslennikova and Lastochkina, 2021) and tomato (Puthiyottil and Akkara 2021).

## 4.5 PGPRs mitigated salinity stress and improved the yield contributing attributes of rice

### 4.5.1 Effective tillers per hill

When rice plants were exposed to 50 and 100 mM NaCl stress, the number of effective tillers hill$^{-1}$ significantly decreased by 55 and 76% compared to the untreated plants, respectively (Figure 13A). Conversely, PGPR treatment increased this metric in salt-stressed rice plants. More specifically, the most significant increase was observed with the application of endophytic *B. subtilis*, at 77 and 171% under 50 and 100 mM NaCl stress, respectively, compared to the stressed plants. Similarly, the epiphytic *B. aryabhattai* showed a similar trend, increasing the number of effective tillers per hill under both levels of saline conditions, by approximately 62 and 171%, respectively. However, the application of endophytic *B. aryabhattai* did not increase the number of effective tillers as significantly as the others did in salt-stressed rice plants.

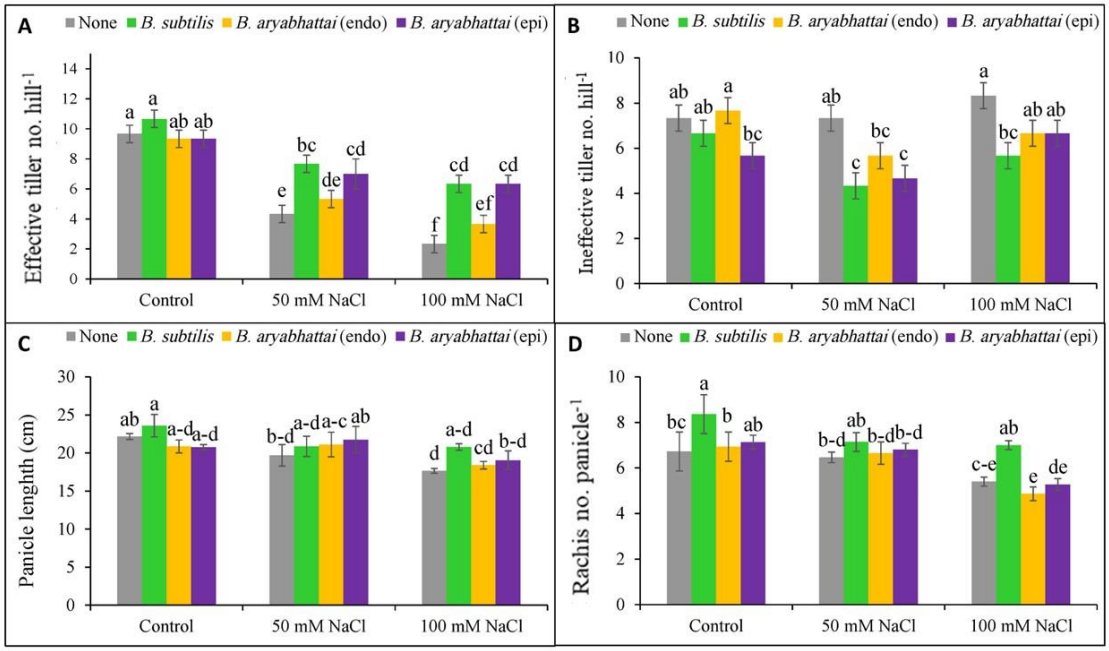


**Figure 13. Variations in effective tiller per no. hill (A), ineffective tiller per no. hill (B), panicle length (C), and rachis no. per panicle (D) of rice (*Oryza sativa* L. cv BRRI dhan100) under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

### 4.5.2 Ineffective tillers per hill

Conversely, when rice plants were subjected to 100 mM NaCl stress, the number of ineffective tillers per hill increased by 14% compared to the untreated plants. No significant change was observed for 50 mM NaCl stress. However, the application of PGPR treatment enhanced this parameter in salt-stressed rice plants. Most notably, the greatest increase was observed with the application of endophytic *B. subtilis*, which resulted in 41 and 32% increases at 50 and 100 mM NaCl stress, respectively, compared to the stressed plants (Figure 13B). Similarly, the epiphytic, *B. aryabhattai*, showed a similar trend in reducing the number of ineffective tillers per hill under both saline

conditions by approximately 36 and 20%, respectively. Interestingly, the level of reduction was nearly identical to that of other PGPRs with the application of endophytic PGPR, *B. aryabhattai*, which also increased the number of effective tillers in salt-stressed rice plants.

### 4.5.3 Panicle length

Salt stress led to a reduction in the panicle length of rice plants, with the most pronounced decrease of 20% observed under 100 mM NaCl stress, twice the reduction seen at 50 mM NaCl stress (Figure 13C). Although the application of PGPRs improved this trait, the results were largely similar, with the exception of a 10 and 18% increase under 50 and 100 mM salt stress, respectively, achieved through the application of epiphytic *B. aryabhattai* and endophytic *B. subtilis*.

### 4.5.4 Rachis number per panicle

The same pattern of reduction was observed with the number of rachis in each panicle of rice plants. The number declined by 4% at 50 mM NaCl stress, but this decrease was amplified nearly five-fold at higher salt concentrations. Despite this, the use of B. subtilis led to increases of 10 and 30% under both saline conditions (Figure 13D). The influence of other PGPRs was relatively similar.

### 4.5.5 Filled grains per panicle

The number of filled grains per panicle in rice plants diminished with increasing salt stress, with the most significant reduction of 58% observed under 100 mM NaCl stress (Figure 14A). Nevertheless, the use of PGPR treatment led to an increase in this parameter, with the most substantial boost seen following the application of endophytic *B. subtilis*, which resulted in increases of 54 and 38% at 50 and 100 mM NaCl stress, respectively, compared to the stressed plants. Similarly, the epiphytic *B. aryabhattai*, followed a similar trend, enhancing the number of filled grains per panicle under both saline conditions by approximately 16 and 27%, respectively.

### 4.5.6 Unfilled grains per panicle

In contrast, when rice plants were subjected to 50 and 100 mM NaCl stress, the number of unfilled grains per panicle increased by 162% and 215%, respectively, compared to the untreated plants (Figure 14B). However, PGPR treatment diminished this increase in salt-stressed rice plants. The greatest reduction was observed with the application of endophytic *B. subtilis* and epiphytic *B. aryabhattai*, which decreased the number of unfilled grains per panicle by 15% at 50 mM NaCl stress and by 24% at 100 mM NaCl stress, respectively, compared to the stressed plants.

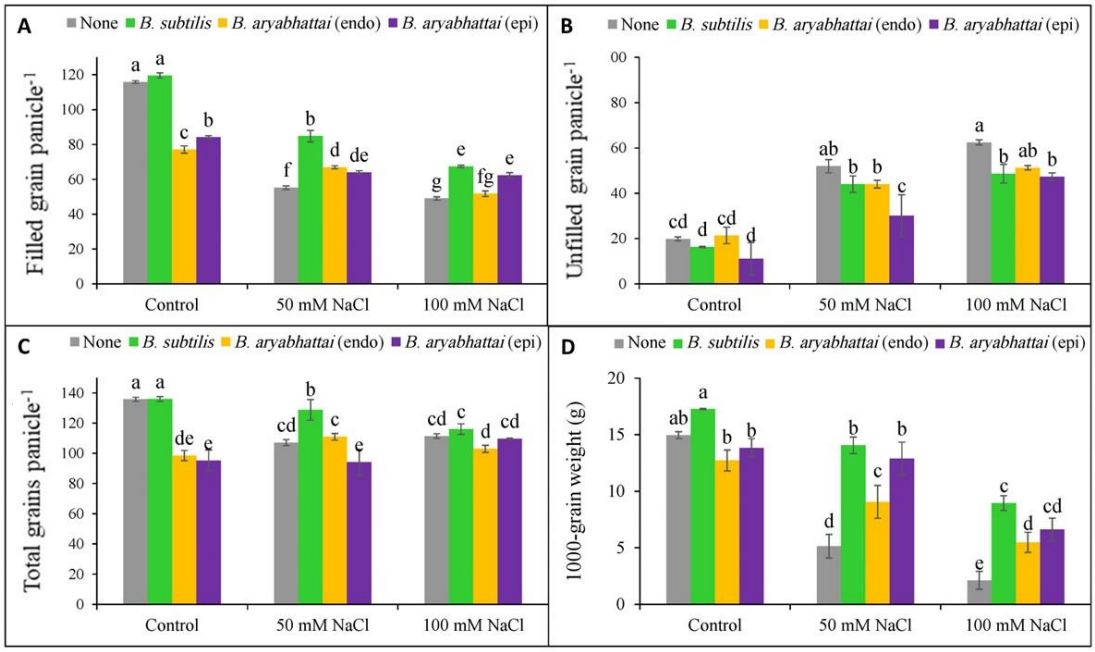


**Figure 14. Variations in filled grain per panicle (A), unfilled grain per panicle (B), total grain per panicle (C) and 1000-grain weight (D) of rice (*Oryza sativa* L. cv BRRI dhan100) under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

### 4.5.7 Total number of grains per panicle

Consequently, the total number of grains per panicle was decreased by 21 and 18% under 50 and 100 mM NaCl stress, respectively, in comparison to the untreated plants (Figure 14C). However, the application of PGPRs reversed this decrease in salt-stressed rice plants. Particularly, the greatest increment of 20% was observed with the use of endophytic PGPR *B. subtilis* at 50 mM NaCl stress, though it proved ineffective at higher salinity levels.

### 4.5.8 1000-grain weight

The weight of 1000 grains also showed variations due to inconsistencies in the number of effective and ineffective tillers under escalating salinity stress levels. Specifically, this parameter diminished by 174 and 86% under 50 and 100 mM NaCl stress, respectively (Figure 14D). However, PGPR treatment ameliorated this decline in salt-stressed rice plants. Notably, the most significant increase was recorded with the application of endophytic *B. subtilis* and epiphytic *B. aryabhattai*, which led to a 174% and 323% surge respectively under 50 mM NaCl, and also a 151% and 231% boost respectively under 100 mM NaCl stress compared to the stressed plants (Figure 14D).

### 4.5.9 Straw yield and grain yield per hill

Owing to the imposition of two distinct levels of salinity stress, both straw and grain yield experienced a considerable decline in rice plants. Specifically, the total straw yield and grain yield dropped by 79 and 69% respectively under 50 mM NaCl stress, and further by 8632

and 89% respectively under 100 mM NaCl stress in comparison to the control plants (Figure 15A, 15B). However, *B. subtilis* ameliorated this condition by enhancing the straw yield by 113% and grain yield by 287% under mild stress conditions. Similarly, other PGPRs also led to significant improvement at lower levels of salinity stress, although their effect was negligible under more severe salinity conditions.

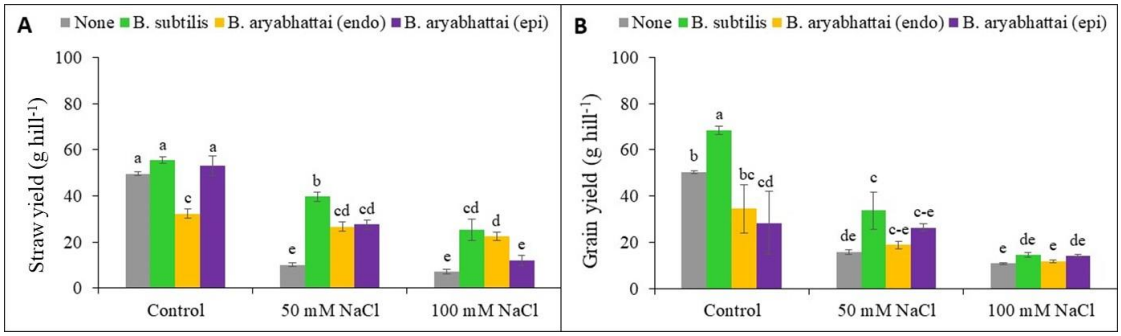


**Figure 15. Variations in straw yield (A) and grain yield (B) of rice (*Oryza sativa* L. cv BRRI dhan100) under varying levels of salt stress (50 mM, and 100 mM NaCl). The plants were treated with endophytic (*Bacillus subtilis*, *B. aryabhattai*) and epiphytic (*B. aryabhattai*) plant growth-promoting rhizobacteria.** Here, the results, presented as bars, depict means (± SD) from three independent experimental sets. The column values were tested for significance ($P \leq 0.05$) using Tukey's HSD test, and distinct letters on the bars signify significant differences between treatments

Salinity significantly compromises plant yield and reproduction, as well as other yield attributes, including causing damage to vegetative growth. This damage is especially evident in rice, where salinity not only reduces yield but also diminishes its nutritional value by causing vegetative shrinkage, altering photosynthetic attributes, creating nutritional imbalance, and reducing the net assimilation rate.

In this study, a considerable reduction in various yield attributes of rice due to salinity stress was observed. These include effective tillers, panicle length, the number of rachis per panicle, filled grain percentage, 1000-grain weight, and straw yield. These findings align with Zheng *et al*. (2019) who discovered salinity lowered grain yield by reducing the number of tillers and 1000-grain weight. This decrease escalated with rising salinity levels.

Kumar and Khare (2016) also observed parallel results, finding that salinity decreased the number of grains per panicle, filled grain percentages, 1000-grain weight, and overall grain yield in both tolerant and sensitive cultivars. Yield reduction was particularly more pronounced in sensitive cultivars.

However, the introduction of PGPR seems to have mitigated the impact of salinity. Plant growth-promoting rhizobacteria improved yield attributes, potentially due to its enhancement of photosynthetic attributes and its ability to limit salt ion accumulation. This improvement aligns with research on PGPR's effectiveness in alleviating salt stress in various crops (Shultana *et al*., 2020).

# CHAPTER V

## SUMMARY AND CONCLUSION

Several attributes of plant growth, physiology, biochemistry, and yield parameters were assessed in this experiment. Salinity stress significantly decreased all the morphophysiological attributes of rice plants, including plant height, FW, DW, LA, photosynthetic parameters, RWC, and EL. Salt stress also induced noticeable oxidative damage and ionic toxicity. Furthermore, a remarkable reduction in the production of the phytohormone IAA was observed.

However, the application of PGPR appeared to ameliorate this situation. It improved nutrient and water uptake, increased IAA synthesis, reestablished ionic homeostasis, and decreased oxidative stress by enhancing the antioxidant system. These changes led to enhanced plant growth and yield.

Upon analyzing all parameters and results, it can be concluded that the PGPRs utilized in this experiment substantially mitigated salt-induced damage in rice plants. Of the endophytic *B. subtilis* and *B. aryabhattai*, the former demonstrated superior performance in mitigating salinity toxicity and enhancing tolerance. Furthermore, the epiphytic *B. aryabhattai* also exhibited positive effects on rice plants under salt stress, following *B. subtilis*. However, these findings pave the way for further research to unravel the intricate mechanisms through which these PGPRs improve salinity tolerance in rice plants and how they might influence grain quality enhancement under salt stress. Also, field experiments can be done with the inoculation of these PGPRs under saline environments in comparison with salt-tolerant rice varieties to find out their effectiveness on yield contributing parameters more precisely.

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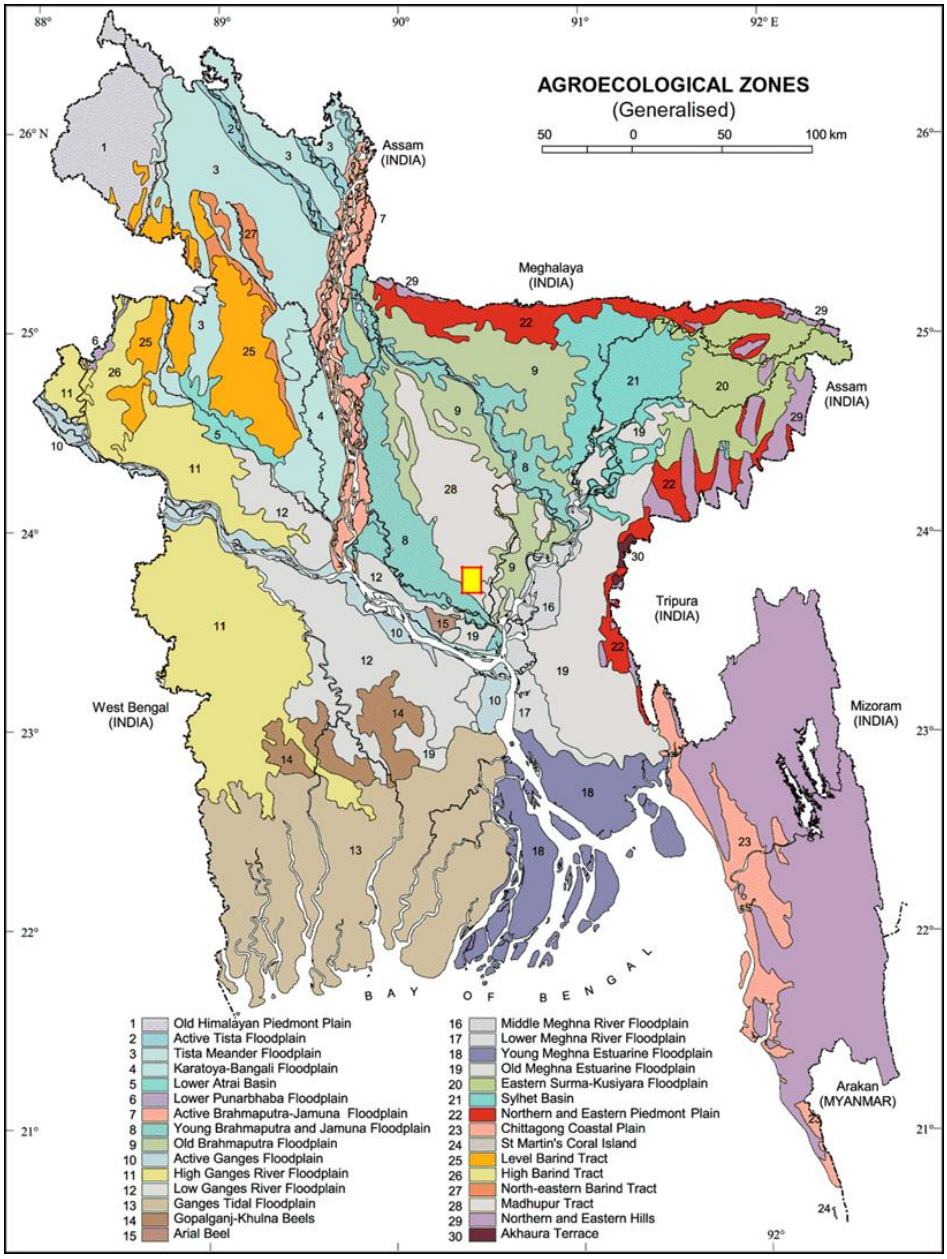
# APPENDICES

**Appendix I.** Experiment location



**Appendix II.** Mean square values and degree of freedom (DF) of plant height, FW, DW and leaf area of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | | |
|---|---|---|---|---|---|
| | | Plant height | FW | DW | LA |
| Treatments | 11 | 107.301 | 51.274 | 0.939 | 29.185 |
| Error | 24 | 10.084 | 0.390 | 0.035 | 1.230 |

**Appendix III.** Mean square values and degree of freedom (DF) of the Chl *a*, Chl *b* and Chl (*a*+*b*) content of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | |
|---|---|---|---|---|
| | | Chl *a* | Chl *b* | Chl (*a*+*b*) |
| Treatments | 11 | 0.059 | 0.293 | 0.592 |
| Error | 24 | 0.003 | 0.004 | 0.007 |

**Appendix IV.** Mean square values and degree of freedom (DF) of the $Na^+$, $K^+$ and $Na^+/K^+$ ratio of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | |
|---|---|---|---|---|
| | | $Na^+$ | $K^+$ | $Na^+/K^+$ |
| Treatments | 11 | 513833.596 | 122651.263 | 0.445 |
| Error | 24 | 792.056 | 4747.222 | 0.002 |

**Appendix V.** Mean square values and degree of freedom (DF) of the SPAD value, $g_s$, $F_v/F_m$ and IAA content of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | | |
|---|---|---|---|---|---|
| | | SPAD value | $g_s$ | $F_v/F_m$ | IAA |
| Treatments | 11 | 17.096 | 13.796 | 0.001 | 11.644 |
| Error | 24 | 5.956 | 1.153 | 0.000 | 0.211 |

**Appendix VI.** Mean square values and degree of freedom (DF) of the RWC, Pro content, MDA content, $H_2O_2$ content and EL of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | | | |
|---|---|---|---|---|---|---|
| | | RWC | Pro | MDA | $H_2O_2$ | EL |
| Treatments | 11 | 307.333 | 5.046 | 246.367 | 70.082 | 163.535 |
| Error | 24 | 9.667 | 0.022 | 0.986 | 0.912 | 0.199 |

**Appendix VII.** Mean square values and degree of freedom (DF) of the AsA content, DHA content, AsA/DHA ratio, GSH content, GSSG content, GSH/GSSG of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | | | | |
|---|---|---|---|---|---|---|---|
| | | AsA | DHA | AsA/ DHA | GSH | GSSG | GSH/ GSSG |
| Treatments | 11 | 2836759.815 | 508368.742 | 4.440 | 118787.052 | 137.182 | 67.172 |
| Error | 24 | 9813.855 | 3249.859 | 0.031 | 685.076 | 1.446 | 0.594 |

**Appendix VIII.** Mean square values and degree of freedom (DF) of the effective tillers per hill, ineffective tillers per hill, panicle length, and rachis per panicle of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | | |
|---|---|---|---|---|---|
| | | Effective tillers per hill | Ineffective tillers per hill | Panicle length | Rachis per panicle[1] |
| Treatments | 11 | 20.515 | 4.414 | 8.659 | 2.812 |
| Error | 24 | 0.389 | 0.333 | 1.229 | 0.229 |

**Appendix IX.** Mean square values and degree of freedom (DF) of the Filled grains per panicle, unfilled grains per panicle, and total number of grains per panicle of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | |
|---|---|---|---|---|
| | | Filled grains per panicle | Unfilled grains per panicle | Total number of grains per panicle |
| Treatments | 11 | 1597.160 | 844.315 | 236.437 |
| Error | 24 | 2.372 | 16.264 | 49.462 |

**Appendix X.** Mean square values and degree of freedom (DF) of the 1000-grain weight, grain yield per hill, and straw yield per hill of rice plants as influenced by PGPR application under different levels of salt stress

| Sthisce of variance | DF | Mean square values | | |
|---|---|---|---|---|
| | | 1000-grain weight | Grain yield per hill | Straw yield per hill |
| Treatments | 11 | 66.326 | 922.812 | 814.731 |
| Error | 24 | 0.857 | 31.419 | 5.648 |