“ROLE OF PLANT GROWTH PROMOTING BACTERIA IN SALT STRESS TOLERANCE IN ORYZA SATIVA (cv. JAYA)”

DISSERTATION SUBMITTED TO GOA UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR

THE DREGREE OF MASTER OF SCIENCE IN BOTANY

BY

MS. GAONKAR SWATI RAMNATH

UNDER THE GUIDANCE OF

DR. SIDDHI K. JALMI

DEPARTMENT OF BOTANY

GOA UNIVERSITY

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CERTIFICATE

This is to certify that this dissertation is a bonafide and an authentic record of this research entitled "**Role of Plant Growth Promoting Bacteria in Salt Stress Tolerance in *Oryza sativa* (cv. Jaya)**" carried out by **Gaonkar Swati Ramnath**, student of Department of Botany, Goa University. This work is carried out under my supervision and guidance at the Department of Botany, Goa University, Taleigao Plateau, Goa, in partial fulfilment for the requirement for the award of **'MASTER OF SCIENCE IN BOTANY'** degree of the University and that no part has been presented before in any other degree or diploma of any University.

Dr. Siddhi K. Jalmi

Dissertation guide

Department of Botany,

Goa University

DECLARATION

I hereby declare that the project entitled **"Role of Plant Growth Promoting Bacteria in Salt Stress Tolerance in *Oryza sativa* (cv. Jaya)"** submitted for the Master of Science in Botany to Goa University is carried out by me under the supervision of Dr. Siddhi K. Jalmi, at the Department of Botany, Goa University. The work is original and had not been submitted in any part or full by me for any other degree or diploma to this or any other university.

Gaonkar Swati Ramnath

Department of Botany

Goa University

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Ms. Swati Gaonkar

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1. <u>INTRODUCTION</u>

Plant growth and productivity are globally being hampered by various biotic and abiotic stresses. Of the others, salinity is regarded as one of the important agricultural problems, as it affects more than 20% of the total irrigated land. Basically, salinity is a form of chemical (abiotic) factor that causes accumulation of soluble salts in the rhizospheric system *(Shultana et al, 2020).* There are five different classes of soil salinity, such as non-saline, slightly saline, moderately saline, strongly saline, and very strongly saline *(Paul and Lade, 2014)*. Among all, less number of salt-tolerable plants can grow in very strongly saline class.

Climate change may lead to even more saline landscapes in many non-irrigated regions because it is accompanied by lesser rainfall and high temperatures in most of the agricultural regions. It will result in a change towards a more arid climate, which is conducive to salt accumulation. Limiting crop losses due to salinity and drought is a major area of concern to cope with the background of increasing food requirements. *(Choudhary et al, 2016).*

Seed germination and early seedling stage are the most salt-sensitive plant growth stages under environmental stresses, because the seedling root is in direct contact with soil and is affected by many soil changes, including salt stress. Many studies have demonstrated that salinity inhibits seed germination of various crops such as wheat, fib bean, rice, maize, and soybean. Moreover, *(Jamil et al, 2005)* observed significant reductions in germination percentage, in germination rate, and in seedling root and shoot lengths of cabbage, sugar beet, paniculate amaranth, and pak-choi *(Choudhary et al, 2016).*

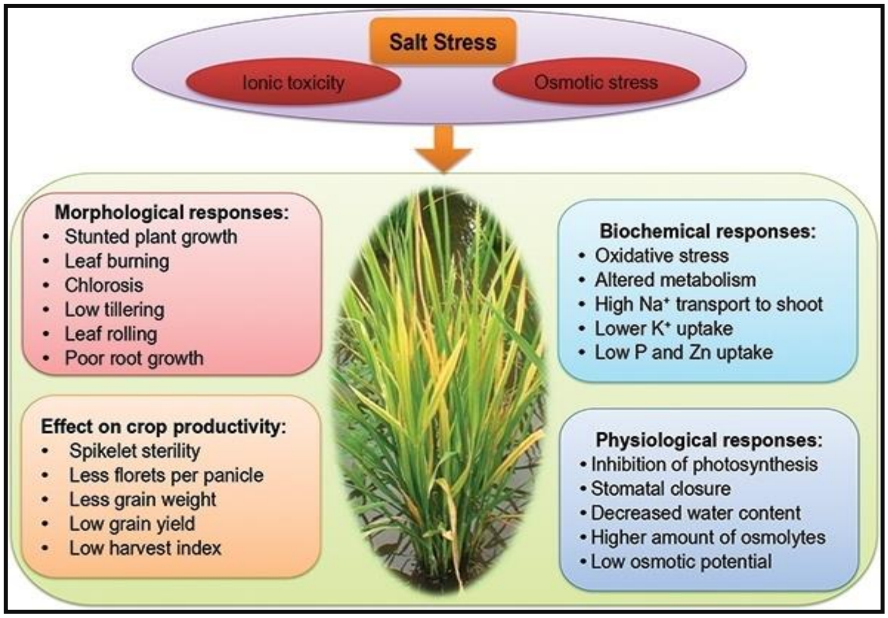

Figure 1.1: Salinity induced major responses in rice plants

Source: *(Rahman et al, 2017)*

Figure 1.2: Fields damaged due to Salinity.

Source: *(George, 2015)*

In India rice is grown in 43.86 million ha, the production level is 104.80 million tones and the productivity is about 2390 kg/ha. It is grown under diver soil and climatic conditions. There is ample scope to increase productivity of rice in the country. Rice is the predominant food crop of Goa occupying an area of 39% (52,442 ha) of the total cultivated land in the state *(Sellappan et al, 2010)*. Popularly rice varieties grown in khazan lands of Goa are Korgut (with awn), Khochro and Assgo. Nearly 18,000 ha all along the sea coast are affected by the coastal salinity through the ingression of sea water *(Bhambure and Kerkar, 2016).*Direct sea water intrusion, through estuaries and the upward movement of salt from shallow water table are the major causes of salinity in salt-affected soils of Goa. Ingression of salt water in fields during monsoon season and its subsequent recession during winter and summer leaves the salt residues behind, which keeps accumulating on the surface due to upward movement and vapour-transpiration.

Studies suggest that as sea levels rise, low-lying coastal areas are increasingly being inundated with saltwater, gradually contaminating the soil. Due to this farmers have given up on farming *(Choudhary et al, 2016), (Mahajan et al, 2015).* Even if these lands are protected from the ingression of sea or creek water by constructing embankments, salts from the shallow water table rises to the surface through capillaries making the surface soils, saline in Goa. *(Bhambure et al, 2016).* Through various breeding methods scientist have created hybrid rice varieties to cop up with this problem however, with this methods has many disadvantage as compared to inbred lines, due to inability of using the seeds form past seasons making it expensive for the farmers to buy the seeds every year. Alternative strategy that is been employed is the use of biofertilizers in place of chemical fertilizers. This microbial formulation of plant growth promoting microbes is known for improving the yields of the crop, vigour and improving the resistance against environmental stressors. Promising measures for improving plant health in saline soils are the use of microbial inoculants, which can ameliorate salt stress, promote plant growth and control diseases. Each plant is associated with specific community of microbiota that is determined by the root exudates secreted by the plant. Hence knowing the microbiota of plant and the beneficial effects exerted by the different microbial communities present will allow us to better formulate the biofertilizer specific for that plant. Also there are some genera soil bacteria that are found to be acting as beneficial for the plant growth, collectively these are referred to as plant growth promoting bacteria (PGPB). PGPB are a group of free living saprophytic bacterial microorganisms that live in the plant rhizosphere and colonize in the root system. They survive in seed or soil, multiply in the spermosphere (soil surrounding the germinating seed) in response to seed exudates rich in carbohydrates and amino acids attach to root surface and become endophytic by colonizing in root cortex region. PGPB generally provides the plant with a compound that is synthesized by the bacterium of facilitating the uptake of nutrients from the environment. Plant growth benefits due to the addition of PGPB include increases in germination rates, root growth, yield including grain, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senescence. *(Sen et al, 2014)*. PGPB can use various mechanisms to stimulate plant growth and development, to protect plants from soil borne diseases, and to increase plant stress tolerance *(Choudhary et al, 2016)*. These mechanisms include, production of phytohormones, antifungal metabolites, and/or lytic enzymes, increasing the availability of plant nutrients, reduction in stress-induced ethylene production, induction of systemic acquired resistance *(Choudhary et al, 2016)*

The utilization of root-associated bacteria that interacts with plants by mitigating stress opens a new advanced technology for combating salinity. Many studies have demonstrated that the use of beneficial microbes can enhance a plant's resistance to adverse environmental stresses, e.g., drought, salinity, nutrient deficiency, and heavy metal contamination. Such inoculants contribute to the development of sustainable agriculture under stressed conditions in plants. *(Choudhary et al, 2016).* The rhizosphere is colonized more intensively by microorganisms than the other regions of the soil because of secretions of the plant through roots and for efficient interaction of microbes with plants. Beneficial rhizobacteria can improve seed germination, root and shoot growth, nutrient uptake, and plant stress tolerance. Moreover, they are able to control various diseases. Several physical and chemical strategies for salinity mitigation have been tested but these methods are not feasible, causing adverse impacts on the ecosystem, thereby creating other problems. Therefore, identifying and developing an eco-friendly strategy that can ameliorate plant growth in response to abiotic stresses is vital in the current agricultural systems *(Shultana et al, 2020).*

Having this in mind, the utilization of PGPB could be the answer to improve rice cultivation on saline soils. The inoculation of seeds of various crop plants, such as tomato, pepper, canola, bean, and lettuce, with PGPB can result in increased root and shoot growth, dry weight, fruit and seed yield and in enhanced tolerance of plants to salt stress.

Since, transgenic technology and molecular breeding are time consuming and labour intensive processes, use of plant growth promoting microbes is gaining wide popularity these days as an alternate strategy for improving stress tolerance of crop plants *(Tiwari et al, 2017).* Among various PGPB genus, *Bacillus* and *Pseudomonas* are most extensively studied rhizobacteria that promote plant growth and development *(Kumar et al, 2014).*

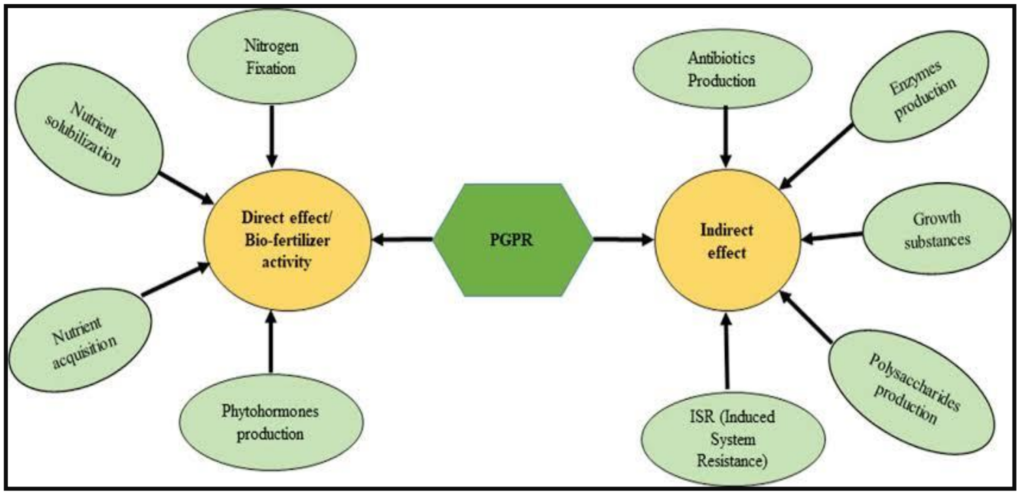

Figure 1.1: Direct and indirect effects of plant growth promoting rhizobacteria on the plant growth

Source: *(Maurya et al, 2019)*

2. ***REVIEW OF LITERATURE***

- A study has demonstrated, inoculating plants with plant-growth promoting rhizobacteria (PGPB) or treating plants with microbe- to-plant signal compounds can be an effective strategy to stimulate crop growth. Furthermore, these strategies can improve crop tolerance for the abiotic stresses (e.g., drought, heat, and salinity) likely to become more frequent as climate change conditions continue to develop *(Backer et al, 2018).*
- Soil Stalinization adversely affects plant growth and has become one of the major limiting factors for crop productivity worldwide. The conventional approach, breeding salt-tolerant plant cultivars, has often failed to efficiently alleviate the situation *(Qin et al, 2016)*
- Hydroponic experiments indicated that the PGPB strain *Bacillus amyloliquefaciens* SQR9 could help maize plants tolerate salt stress. After exposure to salt stress for 20 days, SQR9 significantly promoted the growth of maize seedlings and enhanced the chlorophyll content compared with the control. Additional analysis showed that the involved mechanisms could be the enhanced total soluble sugar content for decreasing cell destruction, improved peroxidase/catalase activity and glutathione content for scavenging reactive oxygen species, and reduced Na^+ levels in the plant to decrease Na^+ toxicity *(Chen at al, 2016).*
- A study investigated the effects of *Azospirillum lipoferum* FK1 strain on chickpea (*Cicerarietinum* L.)Growth and performance under saline conditions (0, 75 and 150 mM NaCl). Salt stress adversely decreased growth, biomass yield, nutrient acquisition, chlorophyll content, gas exchange parameters and total phenolic and flavonoid content of chickpea plants. However, salt stress induced the carotenoid content, osmolytes

level, electrolyte leakage, H_2O_2 content, malondialdehyde level, and levels of enzymatic and non-enzymatic anti-oxidants in chickpea. On the other hand, inoculation of salt-treated chickpea plants with *Azospirillum lipoferum* FK1 significantly improved nutrient acquisition, growth, biomass, photosynthetic pigment synthesis, osmolytes level, gas exchange attributes, phenols and flavonoids content, and enzymatic and non-enzymatic antioxidant levels in chickpea plants when compared to those exposed to NaCl only *(El-Esawi et al, 2019).*

- The role of SN13 was studied in ameliorating various abiotic stresses such as salt, drought, desiccation, heat, cold, and freezing on a popular rice cv. Saryu-52 under hydroponic growth conditions. Apart from this, seedlings were also exogenously supplied with abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA) and ethephon (ET) to study the role of SN13 in phytohormone-induced stress tolerance as well as its role in abiotic and biotic stress cross-talk. All abiotic stresses and phytohormone treatments significantly affected various physiological and biochemical parameters like membrane integrity and osmolyte accumulation. SN13 also positively modulated stress-responsive gene expressions under various abiotic stresses and phytohormone treatments suggesting its multifaceted role in cross-talk among stresses and phytohormones in response to PGPB *(Tiwari et al, 2017).*
- The study included growth parameters, mineral concentration, and antioxidant enzyme level. Salinity reduced plant growth, but PGPB inoculation reduced its harmful effect up to 1% salinity. Plants inoculated with PGPB under saline conditions showed 16% higher germination, 8% higher survival, 27% higher dry weight, and 31% higher plant height. Similarly, PGPB inoculated plants showed increased concentrations of N (26%), P (16%), K (31%), and reduced concentrations of Na (71%) and Ca (36%) as

compared to non-inoculated control plants under saline conditions. Plants inoculated with PGPB under saline conditions also showed significant variations in antioxidant levels and growth physiology. Results suggested that inoculation with PGPB *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* in salt-stressed plants could help to alleviate salt stress in the paddy *(Jha et al, 2013).*

- The physicochemical characteristics such as physical (hulling, head rice recovery(HR), broken rice (BR), grain classification, chalkiness), chemical (alkali spreading value, amylose content (AC), gel consistency (GC), aroma) and cooking characteristics (volume expansion, elongation ratio, water uptake) were studied for 22 traditionally cultivated rice varieties from Goa, in comparison with high yielding rice varieties Jaya, Jyoti and IR8. The hulling percentage ranged from 63-81% and HR recovery from 45-74%. Among the varieties length/breath ratio ranged from 1.5-3.5 and the AC ranged from 14-25%. The lowest percentage of chalkiness was recorded in variety Barik Kudi. Highest GC was recorded in variety Salsi and lowest in Khochro. The kernel elongation ratio ranged from 4.78-1.83 mm and water uptake ratio ranged from 160-390 *(Sellappan et al, 2010).*
- Natural salinity is the result of long-term natural accumulation of salts in the soil or in surface water. Secondary (anthropogenic) salinity results from irrigation and is widely responsible for increasing the concentration of dissolved salts in the soil profile to a level that impairs plant growth and that will result in abandoning agricultural land *(Munns, 2005).*
- Selected PGPB in this study adapted to 6% NaCl stress, were able to enhance plant growth efficiently in the presence of 2% NaCl. This work reports that the isolates selected were able to produce siderophores as well as ACC deaminase enzyme, which

helped the plants to resist salinity stress. T15and C4 showed leading results in protecting tomato plants against salinity stress. T15 showed the lowest NaCl uptake rate as a major mechanism of plant growth promotion. C4 also showed the highest tolerance ratio as a mechanism of plant growth promotion. C5 helped in enhancing the biomass of both the plants, thereby minimizing the toxicity of the stress conditions on plant growth as well as reclamation of salt-affected soil *(Saraf, 2010).*

- Demonstrates that salinity stress induced lower plant fresh dry weight, lower chlorophyll and macro and micro element content in radish plants. The assessment of the effect of salinity on the growth parameters by different salinity levels enabled to conclude that all of the considered parameters were affected by salinity. PGPB seed treatments can ameliorate the deleterious effects of salt stress. The addition of PGPB could offer an economical and simple treatment to salt sensitive radish plants, helping to solve the production problems caused by high salinity *(Yildirim et al, 2008).*
- Screened native bacterial strains of wheat rhizosphere in soils of Varanasi, India, to identify the EPS-producing salt-tolerant rhizobacteria with plant growth-promoting traits. The various rhizobacteria strains were isolated and identified using 16S rDNA sequencing. The plant growth-promoting effect of inoculation of seedlings with these bacterial strains was evaluated under soil salinity conditions in a pot experiment. Eleven bacterial strains which initially showed tolerance up to 80 gL^{-1} NaCl also exhibited an EPS-producing potential. The results suggested that the isolated bacterial strains demonstrated some of the plant growth-promoting traits such as phosphate solubilising ability and production of auxin, proline, reducing sugars, and total soluble sugars. Furthermore, the inoculated wheat plants had an increased biomass compared to the un-inoculated plants *(Upadhyay et al, 2011).*

- Conducted a pot experiment on two wheat genotypes (Aas-11; a salt tolerant and Galaxy-13; salt sensitive) inoculated with *Pseudomonas fluorescence*, *Bacillus pumilus*, and *Exiguobacterium aurantiacum* alone and in consortium. Outcomes of the present study inferred that PGPB employed enhanced compatible solutes accumulation, stimulated potassium and calcium acquisition and reduced antioxidant enzymes activity. These alterations in cellular metabolism ultimately led to the improved growth and yield among both salt tolerant and susceptible varieties under salt stress. However, the salt tolerant variety showed far better growth and yield than the sensitive variety *(Nawaz et al, 2020)*.
- Reports the characterization of two locally-isolated PGPB strains identified as *Bacillus tequilensis* and *Bacillus aryabhattai*, which have shown salt-tolerance and plant growth-promoting characteristics under saline conditions. The plant inoculation test revealed that these strains were capable of increasing the rate of photosynthesis, transpiration and stomatal conductance of three rice varieties, consequently leading to a higher grain yield production *(Shultana et al, 2020)*.

OBJECTIVES

From this background we come to know that plant growth promoting bacteria associated with roots of the plant enhances the growth of the plant and imparts tolerance against the environmental stresses. As salt stress is most detrimental stress for agriculture in Goa due to the high saline soil and the adverse effects on crops, measures are to be taken to combat the salt stress and to increase the crop productivity. PGPB isolated from these saline soils can be a promising biofertilizers to improve the agriculture in such soils. However, this needs understanding of what beneficial effects are exerted by PGPB on physiology of plant and what are the biochemical parameters altered by their interaction. Also the genes regulated by the PGPB in imparting stress resistance will provide us with mechanism of their action. Based on this background, the objectives for the current study were formulated as:

1) **To study the effect of PGPB isolated from the salt pans on plant growth and salt stress tolerance in salt susceptible rice variety (cv. Jaya).**
2) **To study the physiological and biochemical parameters regulated by PGPB under salt stress.**

3. ***MATERIALS& METHODS***

3.1.Plant material and stress treatment

Oryza sativa L. cv. Jaya was used for this entire study. Pilot experiment was carried out to finalise the salt concentration. Three NaCl concentrations were used to induce salt stress, 100mM, 200mM and 300mM. Seeds were sown in different pots and allowed to grow for ten days after which they were treated with different concentrations of salts. The plant height, root length, fresh and dry weight and the overall growth of the plants were examined.

100 mM concentration was selected for the further experiments. The study was carried out by treating the plants with PGPB and then subjecting them to salt stress at 100mM concentration. Pots were divided into 4 groups i) Control, ii) NaCl iii) PGPB, iv) NaCl + PGPB. Soil was collected from a lowland field in Curtorim Goa India. The soil was first autoclaved and then transferred into pots. Seeds were soaked in water for 12 hours and were sown equally in the pots. Pots were kept in 16/8 hours light/dark photoperiod and at temperature of 28ºC - 30ºC. 15 days old plants were inoculated with PGPB and two days later were subjected to salt stress. On day 21 the plants were harvested for conducting various assays.

3.2.Plant Growth Promoting Bacteria (PGPB) Treatment:

Pseudomonas syringae A9 strain of PGPB was procured from American Type Culture Collection (ATCC). PGPB was suspended in Zobel Marine Media and the culture was kept in rotary shaker for 24 hours at 28ºC at 80-100 rpm. The optical density was measured. The suspension culture was then centrifuged at 4000rpm for 20mins. The pellet was dissolved in 0.06% gum acacia solution and 5mL of culture was poured directly into the pots. Gum acacia is a natural emulsifier, stabiliser and texturiser.

3.3.Plant growth analysis:

The shoot length of the seedling was measured from the base of the shoot to the longest leaf tip. The roots were removed carefully from the pots with minimum damage and their mean length from the base of the shoot to the tip of the longest root was measured.

3.3.1. Plant Biomass:

Shoots and roots were weighed separately for fresh weight. Plant samples were then oven dried at 80°C for 24 hours. The dry weight of the whole plant and the root were taken separately.

3.3.2. Determination of Seed Germination:

Rice grains were soaked in 70% ethanol for 5 min in sterile petriplates. The ethanol was discarded and the grains were surface sterilized with 0.5% mercuric chloride for 30s and washed 5 times with sterile distilled water. Rice seeds were soaked in 30mL of the bacterial culture suspension along with 0.06% gum acacia solution and incubated for 30mins. Seeds were placed spaced out in petriplates and allowed to germinate. Germination percentage was calculated after 3 days using the following formula:

Germination percentage= (seeds germinated/total seed)×100

3.4.Plant pigment analysis:

For each treatment, 0.2g of fresh leaf sample was homogenized in 80% acetone, and the solution was centrifuged at 8000rpm for 10mins. The optical density of the supernatant was measured at 663, 645 and 470 nm by a spectrophotometer. Amount of Chl a, Chl b and carotenoids were calculated using the following formula:

- **Chlorophyll a (C_a)= (19.3 × A663–0.86 ×A645) V/100 W**
- **Chlorophyll b (C_b) = (19.3 × A645–3.6 × A663) V/100 W**
- **Total Carotenoids = (1000 ×A470–1.82 × C_a–85.02 × C_b)**

3.5.Protein Estimation:

0.1g of fresh tissue was homogenized using phosphate buffer and the solution was centrifuged at 5000rpm for 20mins. From the supernatant 1mL was taken and the final volume was made to 1mL using distilled water. To this alkaline copper solution was added and incubated for 15mins at room temperature. 0.5M folin's reagent was added to all the test tubes, further incubated for 30mins at room temperature and optical density was measured at 750nm.

4. RESULTS

4.1 Plant growth analysis:

For determining the effect of PGPB (*Pseudomonas syringae)* on the growth promotion of plants under salt stress, different parameters of plant growth and biochemical assays were performed. The assays are given below:

4.1.1 Shoot Length:

For shoot length, 15 days old rice seedlings were treated with NaCl, PGPB and NaCl + PGPB followed by measuring shoot length on day 21.

Results revealed that height was decreased in salt treated plants whereas increased in PGPB treated plants. This increase in height was also observed in plants treated with both NaCl and PGPB together (Table 4.1.1, Fig 4.1.1). The plant height of 26 cm was recorded for NaCl + PGPB treatment which was significantly more than plants treated with NaCl measuring 23 cm. This observation suggested that addition of PGPR ameliorated the negative effects of salt on growth of rice seedling.

4.1.2 Analysis of Root Length:

For root length, 15 days old rice seedlings were treated with NaCl, PGPB and NaCl + PGPB followed by measuring root length on day 21.

PGPB significantly increased the root length of rice seedlings (Table 4.1.2, Fig 4.1.2). Root length of 4.7cm was observed in plants treated with NaCl combined with PGPR and 3.4cm in plants treated with NaCl alone. PGPB produced the highest root length, which was statistically more than root length in NaCl treated plants. The increase in root length observed in rice seedling inoculated with PGPB even in salt stress also suggested that PGPR protected the plant from the negative effects of salt stress. Hence, from this we could observe the beneficial effects of PGPB in ameliorating the salt stress and promoting the plant growth even under the stress.

4.1.3 Analysis of Plant Biomass:

For plant biomass, 15 days old rice seedlings were treated with NaCl, PGPR and NaCl+PGPR and harvested on day 21. Fresh weight and dry weight of shoots and roots were measured separately.

It was observed that treatment with NaCl caused a substantial reduction in the biomass of the plants which was measured to be 134.23 mg as compared to the control plants weighing 220.1 mg, which did not subjected to salt stress (Table 4.2., Figure 4.2.). However, when plant were treated with PGPB prior to salt treatment, the biomass of the plant were not decreased as that of NaCl treated plants and was measured to be 180 mg. Hence, it was conferred that PGPB protected the plant against detrimental effects of salt stress.

4.1.4 Analysis of Seed Germination:

For studying the effect of PGPB on germination of rice, seeds of Jaya variety were grown on MS medium with and without NaCl and in presence and absence of PGPB. The germination percentage were calculated by comparing the germination of seeds grown on MS with NaCl to seeds prior inoculated with PGPB and grown on MS with NaCl medium.

The germination of seeds was greatly affected by the salt stress which showed only 27.78% of germination. However, the seeds prior inoculated with PGPB showed improved germination of seeds to 55.55% even under salt stress. The PGPB alone also promoted the germination of rice seeds showing the highest percentage of germination compared to the seeds grown without PGPB (Table 4.1.4, Figure 4.1.4). Hence from this experiment it was suggested that PGPB not only helped in alleviating the detrimental effect of salt stress on germination but also promoted germination in control seeds which were not under salt stress.

4.1.5 Pot experiment

This experiment was conducted to study the overall growth of salt susceptible rice variety Jaya when treated with NaCl (100mM) and the beneficial effect of PGPB *P. syringae* in mitigating the effects of slat stress. It was observed that plants under salt treatment showed less growth however treating them with PGPB prior to salt stress showed better tolerance towards salt stress (Fig 4.1.6).

4.2 Biochemical analysis:

4.2.1 Plant pigment analysis:

The major pigments of plants important for normal growth and development and for photosynthesis are Chlorophyll A Cholrphyll B and carotenoids. The content of these gets changed in plant under stress; hence these parameters were studied in rice variety Jaya under salt stress, with presence and absence of PGPB. For pigment analysis, 15 days old rice seedlings were treated with NaCl, PGPB and NaCl+PGPB followed by harvesting them on day 21.

Results revealed that salt stress led to a significant reduction of the carotenoid and chlorophyll contents (Table 4.2.1, Figure 4.2.1) as compared to control plants without any treatment. However salt stress treatment in PGPB inoculated plants showed increased carotenoid as well as Chlorophyll content of plants as compared to plants treated with NaCl alone.

4.2.2 Protein Estimation

Protein content is necessary for normal growth and development of any living organism. Its estimation also becomes important to know if the plant is under any stress. The content of protein becomes low when plant is under stressful condition and this might be due to oxidation of proteins by reactive oxygen species generated in stress. For protein estimation 15 days old rice seedlings were treated with NaCl, PGPB and NaCl+PGPB followed by harvesting them on day 21.

Protein content was significantly decreased in plants subjected to salt stress as compared to control plants without any salt treatment. However, inoculation of PGPB in plants prior to salt stress showed increase in protein content as compared to the plants under salt stress. This suggested the beneficial effect of PGPB in protecting the plant and mitigating the salt stress (Table 4.2.2 And Figure 4.2.2).

Table 4.1.1 Effect of NaCl and PGPB on shoot length of rice seedlings.

SAMPLES	SHOOT LENGTH (cm)
Control	26.167 ± 1.0408
NaCl	21.500 ± 2.1794
PGPR	25.627 ± 0.5001
NaCl+PGPR	25.200 ± 0.9165

Table 4.1.2 Effect of NaCl and PGPB on root length of rice seedlings.

SAMPLES	ROOT LENGTH (cm)
Control	7.600 ± 0.4359
NaCl	3.400 ± 0.1000
PGPR	6.200 ± 0.5292
NaCl+PGPR	4.700 ± 0.1000

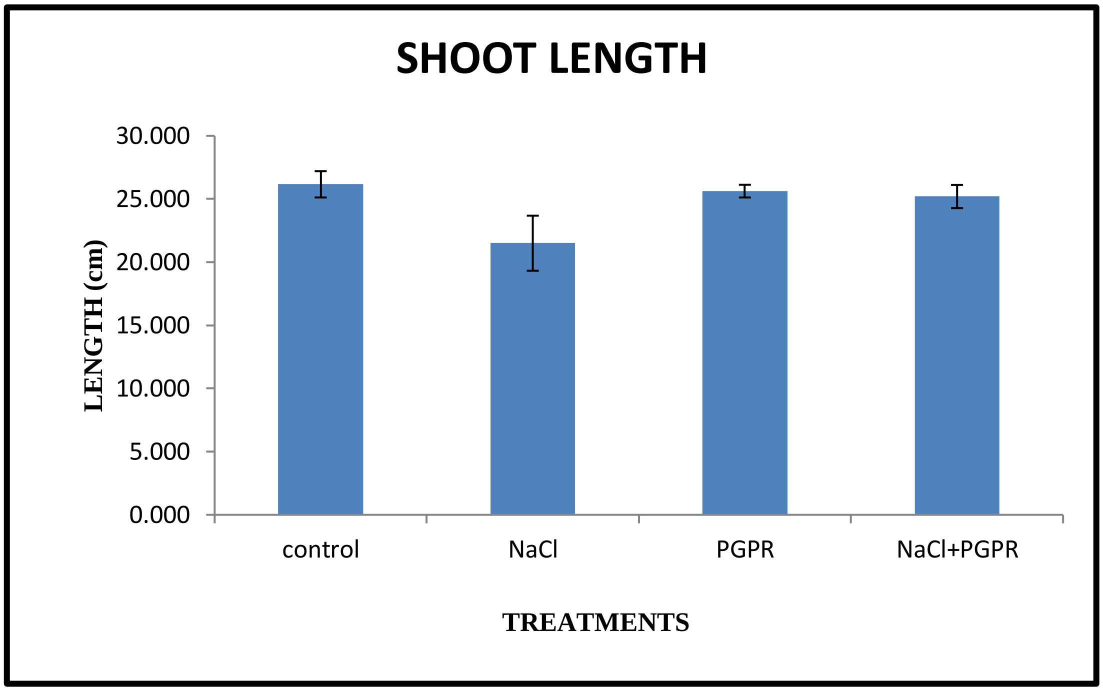

Figure 4.1.1. Effect of NaCl and PGPB on shoot length of rice seedlings.

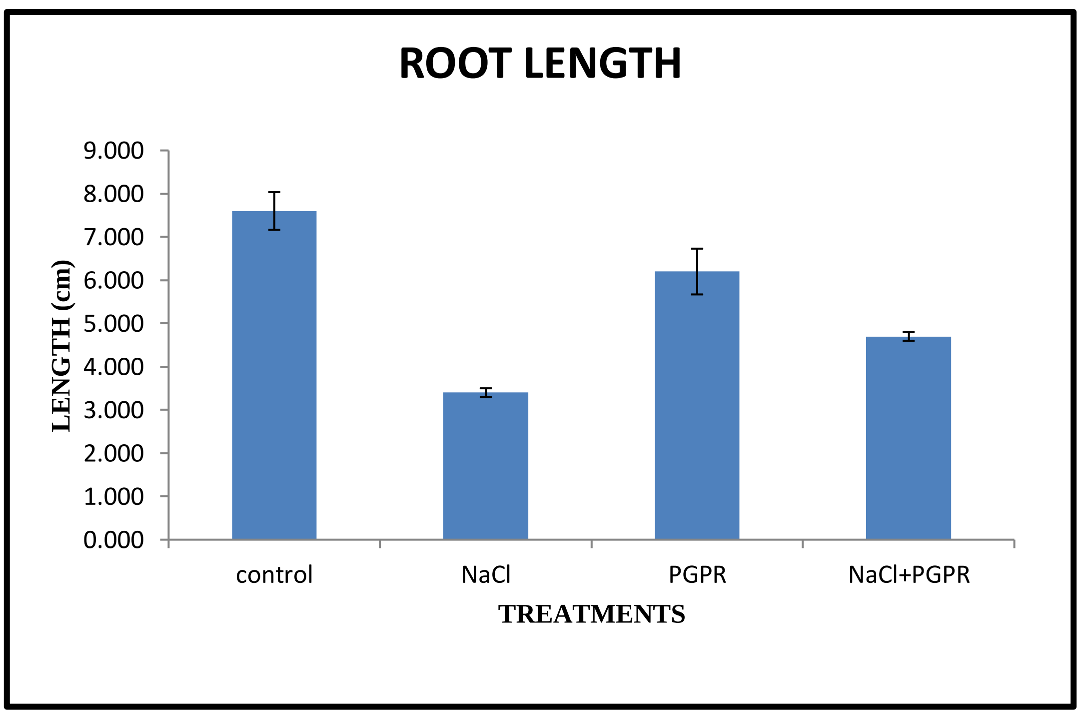

Figure 4.1.2. Effect of NaCl and PGPB on root length of rice seedlings.

Table 4.1.3 Effect of NaCl and PGPB on biomass of rice seedlings.

SAMPLE	BIOMASS in mg
CONTROL	220.1 ± 13.937
NaCl	134.233 ± 14.231
PGPR	179.23 ± 7.735
NaCl+PGPR	180.063 ± 3.147

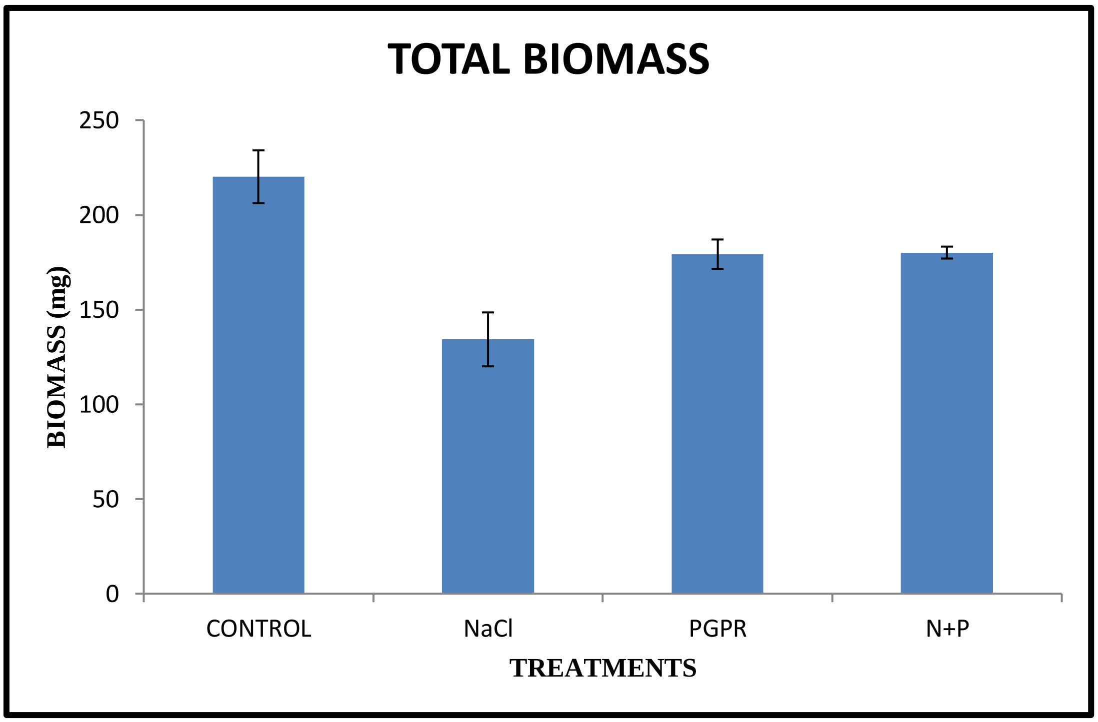

Figure 4.1.3. Effect of NaCl and PGPB on biomass of rice seedlings.

Table 4.1.4 Effect of NaCl and PGPB on germination percentage of rice seeds.

SAMPLES	GERMINATION PERCENTAGE (%)
Control	85.1767 ± 9.0712
PGPR	27.775 ± 9.2952
NaCl	79.6217 ± 8.3633
NaCl+PGPR	55.55 ± 9.9370

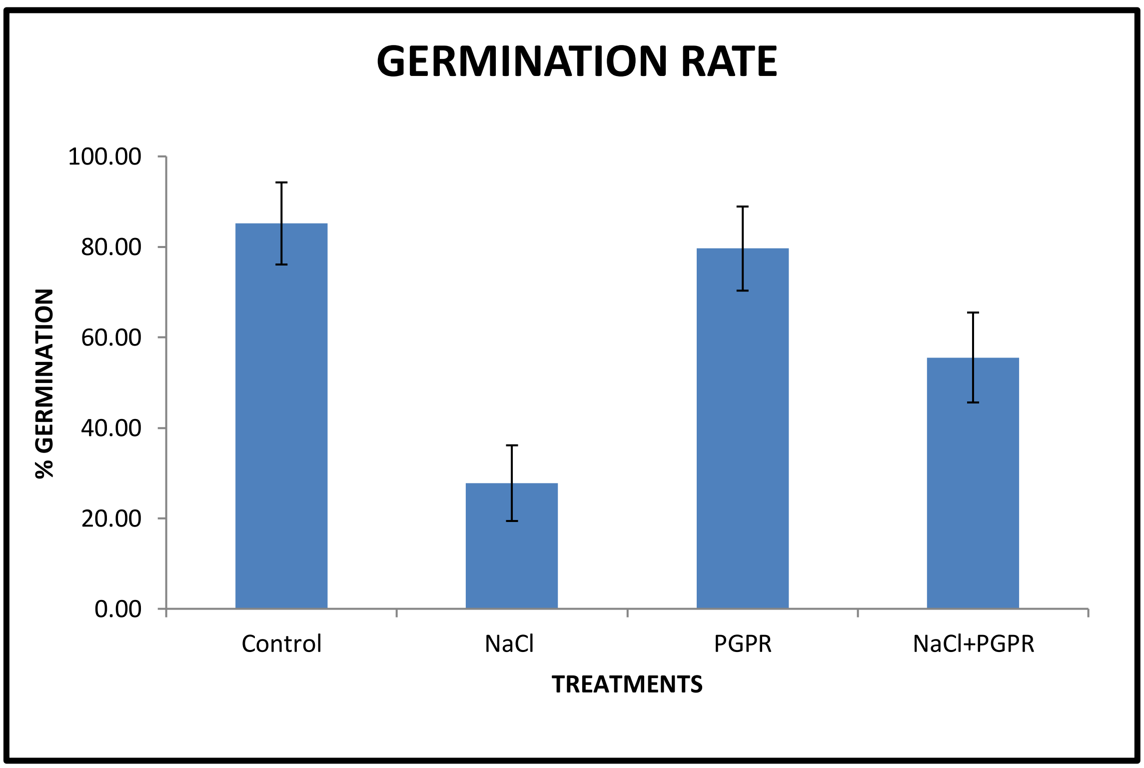

Figure 4.1.4 Effect of NaCl and PGPB on germination percentage of rice seeds.

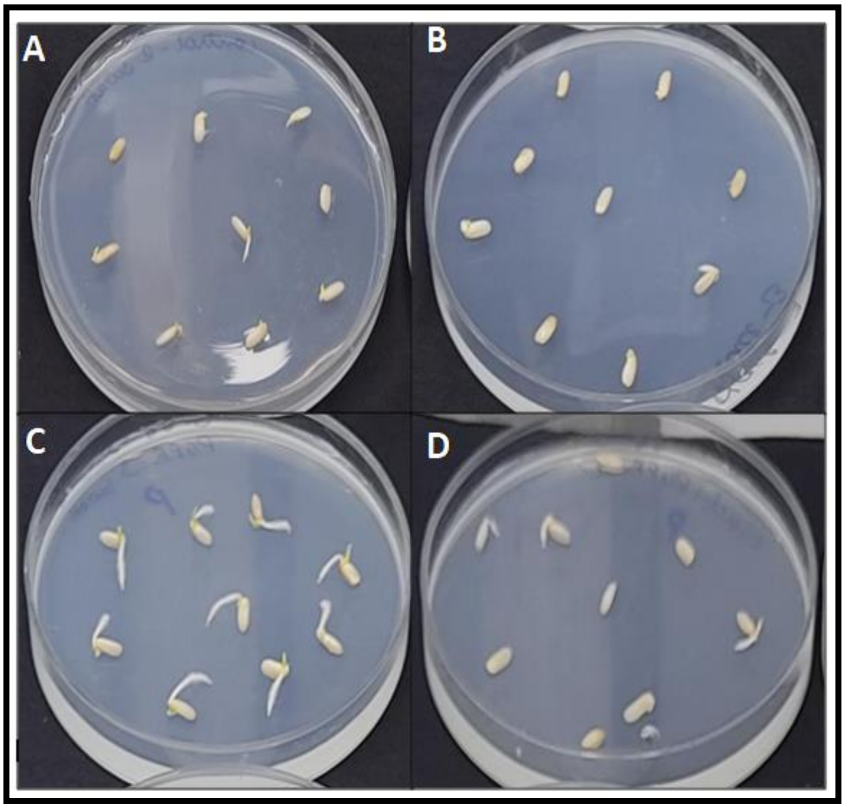

Figure 4.1.5: Germination analysis for the rice seeds on MS medium, treated with B: NaCl, C: PGPB, D: PGPB+NaCl. A: Control without any treatment.

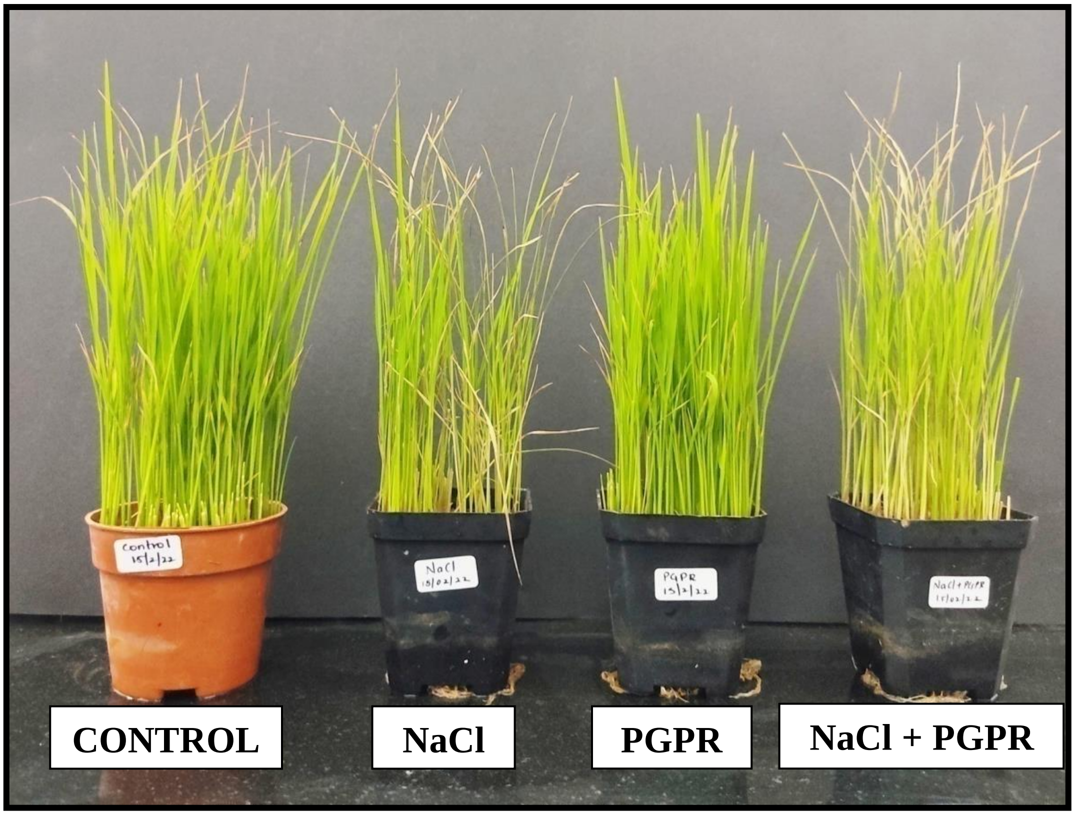

Figure 4.1.6: Pot experiment showing plants subjected to salt stress and alleviation of salt stress by PGPB treatment

Table 4.2.1 Effect of NaCl and PGPB on Pigment contents of rice seedlings.

SAMPLES	CHL A (mg/mL)	CHL B (mg/mL)	CAROTENOIDS (mg/mL)
control	14.295 ± 1.449	13.702 ± 0.542	10.624 ± 0.762
NaCl	1.960 ± 0.378	5.752 ± 1.643	3.290 ± 0.484
NaCl + PGPB	6.607 ± 0.649	5.636 ± 0.266	8.122 ± 0.328

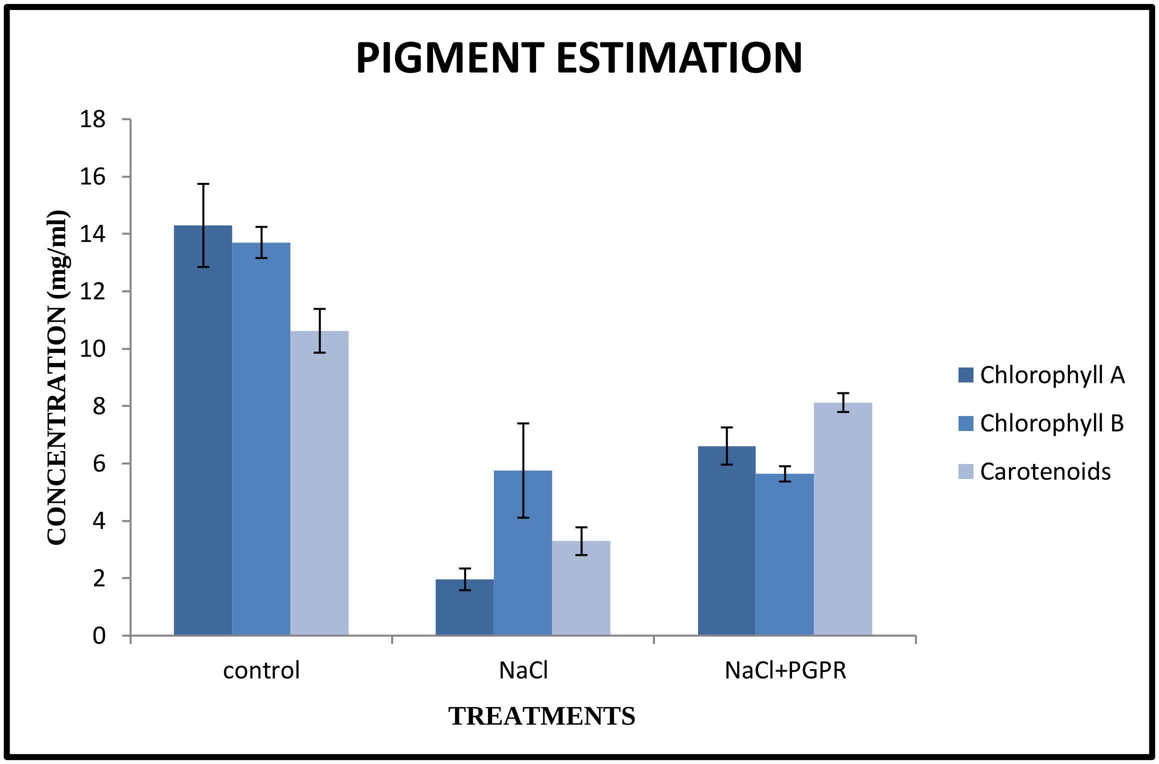

Figure 4.2.1 Effect of NaCl and PGPB on Pigment contents of rice seedlings.

Table 4.2.2 Effect of NaCl and PGPB on protein content of rice seedlings.

SAMPLE	PROTEIN CONTENT (mg/mol)
Control	67.482 ± 1.946
PGPR	57.983 ± 4.297
NaCl	24.592 ± 4.807
NaCl + PGPR	43.648 ± 2.449

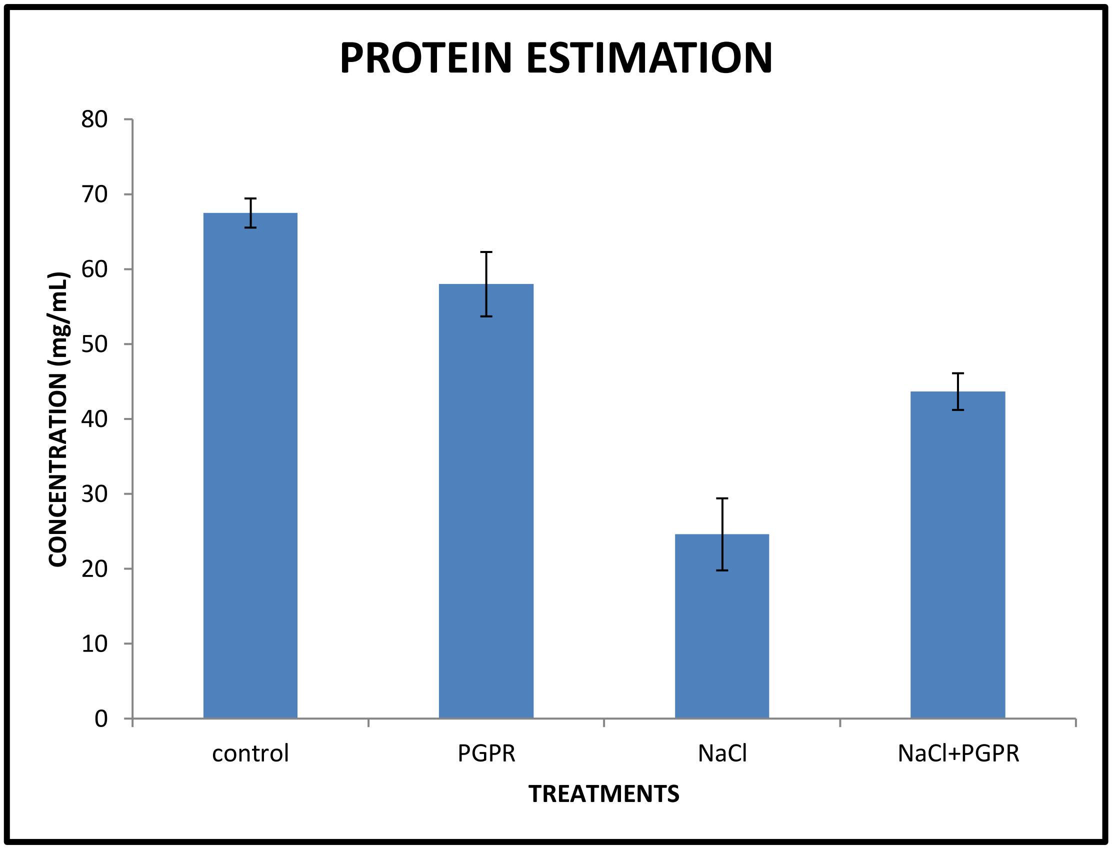

Figure 4.2.2 Effect of NaCl and PGPB on Protein content of rice seedlings.

5. DISCUSSION

Soil salinity is a prevalent environmental restraint to agriculture productivity and food security. Salt stress is responsible for 20– 50% yield loss of important agricultural commodities including wheat, rice and maize around the world. So, there is a pressing need to adapt new sustainable approaches in addition to the use of organic or inorganic soil amendments along with salt resistant crop varieties to improve the productivity of such problematic soils *(Egamberdieva et al, 2014)*.

PGPB play a key role in enhancing plant growth and crop tolerance to environmental stresses by regulating the main growth-promoting pathway in plants. In the current investigation, high salinity reduced the growth of rice plants and biomass yields which are in line with previous findings in different crops, including wheat *(Kausar et al, 2013)*, tomato *(Abdel Latef and Chaoxing, 2011)*, pepper *(Abdel Latef and Chaoxing, 2014)*, and chickpea *(Ahmad et al, 2016)*. However, under saline conditions, rice plants inoculated with PGPB showed improved growth traits compared to non-inoculated plants. Treatment of PGPB was found better in improving the plant height significantly over control. $CaCl_2$ has positive effect on ameliorating adverse effects of salt stress. This was supported by *(Afzal et al, 2015)* who explained that seed treatment with PGPB would counteract the salinity induced growth inhibition in wheat seedlings.

In this study, we investigated the effectiveness of PGPB in mitigating the detrimental effects of salt stress, whether they could protect the salt susceptible rice variety treated with salt stress. As compared to NaCl treated plants, plants treated with PGPB prior to salt treatment showed better growth and salt stress tolerance in all the studied aspects.

Salinity mitigated chlorophyll levels in rice plants in the present investigation. This could be due to the stimulation of salinity stimulated chlorophyllase that degrades pigments protein, resulting in decreased chlorophylls synthesis and low chlorophyll levels *(Abd_Allah et al, 2018)*. The results are in harmony with previous studies that indicated low chlorophyll content due to salt stress on different crops, including *Cicer arietinum (Rasool et al, 2013), (Abd_Allah et al, 2018)* and *Ephedra alata (Alqarawi et al, 2014)*. Carotenoids synthesis was enhanced when plants were treated with PGPB under salinity stress. Inoculation of rice plants with PGPB alleviated the salt induced deleterious effects by enhancing the photosynthetic pigments synthesis. These findings are in harmony with previous studies which indicated a significantly enhanced growth of *Cicer arietinum*, *Raphanus sativus* and runner bean because of their inoculations with PGPB that stimulated the photosynthetic pigments synthesis *(Stefan et al, 2013); (Mohamed and Gomaa, 2012); (Abd_Allah et al, 2018).*

In the present study, high salinity induced decreased protein content in rice plants. However, PGPB treated plants showed significant increased in protein content under salt stress as compared to non inoculated plants under salt stress. This was in line with inoculation of salt tolerant *Bacillus amyloliquefaciens* SN13 onto rice *(Oryza sativa)* plants exposed to salinity (200 mM NaCl) in hydroponic and soil conditions increased plant salt tolerance and affected expression of 14 genes, of which, four (SOS1, ethylene responsive element binding proteins EREBP, somatic embryogenesis receptor-like kinase SERK1 and NADP-malic enzyme NADP-Me2) were up regulated *(Nautiyal et al, 2013)*. In the current study, the beneficial effects and stress tolerance imparted by PGPB on rice plants under salt stress might be due to providing essential nutrients and plant growth hormones, needed by the plants under stress to get restored. Also, it is assumed that PGPB can modulate various stress responsive genes encoding important enzymes and transcription factors needed for tolerating the salt stress. The very important enzymes that might be modulated by the treatment of PGPB are superoxide dismutase (SOD), PCS5 (involved in proline biosynthesis), Protein kinases like MAPKs, CDPKs, etc and the important transcription factors are DREB, EREBF, WRKY, etc. The study further will focus on understanding the genes that are regulated by the PGPB *P. syringae*. The regulation of these genes will further throw light on the mechanism by which this PGPB helps plants to ameliorate the salt stress.

6. CONCLUSION

High salinity adversely affected the growth, biomass, germination percentage, photosynthetic pigment contents and protein content of rice plants, thereby altered their physiological, biochemical and molecular processes. These alterations might be due to the induction of osmotic stress and ionic toxicity in the plants under saline conditions, causing plant growth reduction via restricting ion homeostasis, photosynthesis and nutrient uptake *(Abd_Allah et al, 2018).*Additionally, the excessive generation of toxic Reactive oxygen species (ROS) induced by high salinity hinders metabolism, negatively influences cell functional integrity and causes oxidation of cell molecules such as proteins, lipids and chlorophyll *(Ahmad et al, 2016).* On the other hand, PGPB inoculation improved plant growth and alleviated the inhibitory impacts of salt stress on rice plants.

The reality of abiotic stress in reducing the availability of food for the growing human population is obvious. The temperature of the atmosphere is rising and deviation or instability in rainfall is frequently observed in our environment today. This creates a tension in the sustainability of farming practice for food production *(Enebe et al, 2018).* And as human beings devise an alternative to combat these challenges by adopting irrigation methods, salinity becomes the end product of this alternative practice. This also affects plants negatively. Plants own defense system is not enough to overcome these detrimental stressed conditions. Significant effects of plant tolerance to abiotic stress are that it will result in promoting yield and production of crops to feed humans and livestock. This can be achieved via the search, selection, and engineering of plant species capable of resisting salinity stress. The use of plant growth-promoting will go a long way in supporting the plant to develop both intrinsic and extrinsic ability to tolerate stressful conditions and sustain yield. A future study involving the field trial on the affected areas is crucial to ensure that the locally-isolated strains can survive the soil condition and are compatible with the conventional farmer practices.

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