

# ENHANCING CROP SALINE STRESS TOLERANCE AND SOIL REMEDIATION WITH PHYTOBENEFICIAL BACTERIA: DIVERSE APPROACHES FOR IMPROVEMENT

## ABSTRACT

Soil salinity, a pervasive issue exacerbated by factors like irrigation and climate change, poses a significant threat to global food security. The accumulation of salts not only hampers crop growth and yield but also jeopardizes the livelihoods of millions who depend on agriculture for sustenance. Elevated salt levels in saline soils induce osmotic, ionic, oxidative, and water stress in plants. Implementing biological solutions offers the most dependable and sustainable method to safeguard food security while reducing reliance on agrochemicals which hampers various physiological and metabolic processes in plants. To ensure optimal plant growth under such changing conditions, implementing biological solutions (Rhizobacteria) offers the most dependable and sustainable method to safeguard food security while reducing reliance on agrochemicals must be integrated into agricultural practices. This chapter concisely explores the mechanisms and utilization of beneficial microorganisms in both plants and soil to mitigate salt stress. It also addresses the current limitations and suggests potential areas for improvement in future research.

Keywords: Saline soil, Rhizobacteria, Remediation, *Pseudomonas* spp.

## INTRODUCTION

The population of Earth reached 8.1 billion people in 2010; if growth continues at its current rate, that number is predicted to reach 9.7 billion people by 2050 (Projections of population growth). Additionally, water, air and soil pollution are responsible for about 40% of deaths globally and environmental deterioration like this, together with population growth, are thought to be important factors in the rapid rise in human disease worldwide. Various abiotic factors such as temperature, salinity, drought, pesticide and fertilizer usage, soil pH, and heavy metal contamination can impede crop productivity (Yadav et al., 2020; Kumar 2020; Ahmad et al., 2011). Out of all of these, soil salinity's worldwide effect on crop productivity has emerged as a major barrier. Human activity has increased the development

of soil salinization during the last few decades (Lambers 2003; Bargaz et al. 2018; Sultana et al. 2020). Important soil activities like respiration, residue breakdown, nitrification, denitrification, soil biodiversity, and microbial activity are all impacted by the extreme soil salinization (Schirawski & Perlin, 2018). Increased soil salinity and decreased crop output are also observed in areas with excessive fertiliser application (Rütting et al., 2018). The technique of removing salt from saline soil is labor-intensive and expensive (Qadir et al., 2014). For quite some time, the rehabilitation of saline soils has primarily relied on physical and chemical techniques. Within the realm of physical processes, soluble salts within the root zone are extracted through methods such as scraping, flushing, and leaching (Ayyam et al., 2019). Nevertheless, chemical methods often involve the utilization of gypsum and lime as neutralizing agents to mitigate saline soil conditions (Keren, 2005). However, these methods are deemed unsustainable and are considered inefficient, particularly when the salt concentration reaches excessively high levels. The common practice of cultivating salt-tolerant crop varieties, such as barley and canola, on saline soils is widespread (Fita et al., 2015). Nevertheless, due to their limited salt tolerance profile, these crops have a restricted global distribution and cannot be effectively utilized in soils with moderate to high electrical conductivity (EC) levels (Morton et al., 2019) also highlighted that despite vigorous efforts from the research community, only few salt tolerance genes have been identified having real applications in improving productivity of saline soils.

Therefore, achieving viable crop yields in saline soils is imperative. In addition to utilizing salt-tolerant varieties or chemical neutralization methods, it's essential to incorporate sustainable approaches. In the last few years, research showed that the use of salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) and halotolerant rhizobacteria (HT-rhizobacteria) in saline agriculture can be harnessed for enhancing productivity and improving soil fertility as well (Grover et al., 2011). As they significantly impact biogeochemical cycles, soil fertility, and plant health, they play a crucial role in influencing plant growth and the uptake of nutrients. This review critically examines the role of salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) and halo tolerant rhizobacteria (HT-rhizobacteria) in responding to salt-affected soil and their beneficial effects on key crops. It delves into their mechanisms for remediating salt-affected soil under diverse environmental conditions. The present review focuses on the enhancement of productivity under stressed conditions and increased resistance of plants against salinity stress by application of plant growth promoting microorganisms.

The utilization of Plant Growth-Promoting Rhizobacteria (PGPR) has been expanded to remediate contaminated soils in conjunction with plants. Therefore, there is a pressing necessity to augment the effectiveness of limited external inputs by optimizing the combinations of beneficial bacteria within sustainable agricultural production systems. This review delves into the significance of soil-beneficial bacteria and their contributions to promoting plant growth through both direct and indirect mechanisms. A deeper understanding of these varied mechanisms will contribute to establishing these bacteria as invaluable allies in the future of agriculture.

## **1. MECHANISIM OF CROP SALINE STRESS TOLERANCE BY SOIL BENEFICIAL BACTERIA**

Soil salinity poses a significant challenge for irrigated agriculture. In hot and arid regions across the globe, soils often exhibit high salinity levels, resulting in limited agricultural productivity. It's worth noting that all soils inherently contain some amount of water-soluble salts(Shrivastava & Kumar, 2015). Soluble salts are a form of essential nutrients that plants absorb; nevertheless, an overabundance of them can seriously impede plant growth. Global natural resources have suffered greatly as a result of land degradation processes over the past century, whether they are physical, chemical, or biological. Compacted soil, contamination from both organic and inorganic sources, and a decrease in microbial variety and activity are a few of these problems Patel and Dave 2011).(Bidalia et al., 2019)(S. Singh & Singh, 2022). Salinity destructively interrupts the physical and chemical properties of soil as well as affects crop growth to a higher extent (K. Singh, 2016).

To address this issue, beneficial microorganisms called plant growth-promoting rhizobacteria (PGPR) could serve a vital function. These rhizospheric bacteria have the ability to efficiently colonize plant roots, thereby contributing to soil fertility maintenance. They provide a promising alternative to traditional inorganic fertilizers and pesticides(Majeed et al., 2015). Previous reports have highlighted the efficacy of PGPR in enhancing the growth of different crops under conditions of salt stress(Cardinale et al., 2015); (Soldan et al., 2019).The initial selection of locally-isolated salt-tolerant PGPR for addressing salinity is essential to guarantee their effectiveness. Studies have shown that indigenous strains are more proficient in enhancing plant resistance to salinity stress compared to PGPR from non-saline ecosystems(Etesami & Beattie, 2017); (Egamberdieva & Kucharova, 2009).These beneficial microbes employ various mechanisms to mitigate salt stress, such as regulating the

Na<sup>+</sup>/K<sup>+</sup> ratio by secreting extracellular polymeric substances known as exopolysaccharides (EPS), this mechanism enhances their survival in unfavorable soil conditions(R. P. Singh & Jha, 2016)(Vurukonda et al., 2016).

The previous findings have reported that several bacterial genera, including *Pseudomonas*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Planococcus*, *Halomonas* could produce EPS (Exopolysaccharides) in salt stress condition (Upadhyay et al., 2011)(Qurashi & Sabri, 2012).The exopolysaccharides play a vital role in bacterial aggregation or flocculation, chelates the various cations including Na<sup>+</sup> (Watanabe et al., 2003)(Nunkaew et al., 2015), facilitating the production of yield, this process involves the specific adsorption of the polymeric segment and polymer bridging between cells(Tenney & Stumm, 1965)(M. Arora et al., 2010). Additionally, EPS are highly beneficial in the formation of bacterial biofilms and enhancing bacterial colonization on plant root surfaces(Y. Chen et al., 2013).Exopolysaccharides are able to lessen the hostile effect of osmotic-stress by augmenting fresh weight, dry weight and water content in plants (Ghosh et al., 2019). In addition to that, PGPR are able to produce multiple plant growth-promoting properties such as indole acetic acid production, biological nitrogen fixation, solubilization of soil phosphorus (P) and potassium (K), and production of siderophores and hydrolyzing enzymes under salt stress condition (Kang et al., 2009)(Richardson et al., 2009).(Yousef, 2018)(Goswami et al., 2014).Plants treated with Exo-poly saccharides (EPS) producing bacteria display increased resistance to water and salinity stress due to improved soil structure (Sandhya et al., 2009). EPS can also bind to cations including Na<sup>+</sup> thus making it unavailable to plants under saline conditions.

The SEM observations supported all these salt-tolerance attributes, revealing the bacterial capacity to produce EPS, facilitate flocculation, and form biofilms when subjected to saline conditions compared to non-saline environments. Bacterial cells were observed to associate with the plant root system, notably enhancing moisture retention capacity and bolstering the defense system against various abiotic stresses. Previous research also noted a reduction in bacterial EPS and biofilm formation with increased NaCl concentration (Havasi et al., 2008). The detrimental effects of salinity can be mitigated through the application of salt-tolerant PGPR, as demonstrated in this greenhouse trial. This intervention notably enhanced the photosynthesis of all three rice varieties, resulting in increased grain yield under saline conditions (Shultana et al., 2020)(Tewari & Arora, 2014).Soils experiencing salt stress

are recognized for their ability to inhibit plant growth (Paul, 2012). In their natural habitat, plants are colonized by both endocellular and intracellular microorganisms (Gray & Smith, 2005). The rhizosphere microorganisms, especially beneficial bacteria and fungi, have the potential to enhance plant performance in stressful environments, thereby directly and indirectly improving yields (Dimkpa et al., 2009). Certain PGPR can directly stimulate plant growth and development by supplying fixed nitrogen, phytohormones, iron sequestered by bacterial siderophores, and soluble phosphate. Others indirectly benefit plants by protecting them against soil-borne diseases, primarily caused by pathogenic fungi (Lugtenberg & Kamilova, 2009). Soil salinization presents a significant challenge to agricultural productivity worldwide. Crops cultivated in saline soils face issues such as high osmotic stress, nutritional imbalances and toxicities, poor soil structure, and decreased crop yields. Studies have confirmed that salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) are capable of producing various phytohormones, including auxins, gibberellins, and cytokinins (Dodd et al., 2010). Additionally, they synthesize ACC deaminase (Glick, 2004), secondary compounds such as exopolysaccharides (Upadhyay et al., 2011; Timmusk et al., 2014) and osmolytes (proline, trehalose, and glycine betaines) (Bano and Fatima, 2009; Upadhyay & Singh, 2015). Furthermore, these bacteria play a role in regulating plant defense systems and activating the plant's antioxidative enzymes under salt stress (Hashem et al., 2016; Ali et al., 2022).

Numerous studies have highlighted potential mechanisms of salt tolerance in PGPR, particularly when they function as endophytes. However, it's important to note that only a small portion of these beneficial bacteria are able to penetrate the root cell, and the interaction between plants and microbes primarily takes place within the 5mm rhizosphere zone (Shultana et al., 2020). Salt-tolerant bacterial strains have demonstrated elevated nitrogenase activity in saline environments and possess the capability to synthesize osmolytes. These osmolytes help maintain cell turgidity and support metabolism in adverse conditions. (Yan et al., 2015; Kumar et al., 1999).

Salinity-induced nutritional imbalance poses a challenge to plant growth and productivity, particularly affecting phosphorus (P) uptake and transport. To address P deficiency in salt-affected soils, P fertilizers are commonly recommended. However, employing salt-tolerant P-solubilizing rhizobacteria can significantly enhance P availability in saline soils (Salwan et al., 2019). In recent studies, plant growth-promoting (PGP) bacteria

have been discovered to boost plant tolerance to salinity, particularly those bacteria that are associated with plants (Glick, 2004). Endophytic actinomycetes are particularly intriguing due to their dual role. Not only do they produce various bioactive secondary metabolites, safeguarding plants against infectious diseases (Misk & Franco, 2011), but they also exhibit the capacity to enhance plant growth. They carry several plant growth-promoting (PGP) traits, including the production of siderophores for iron acquisition, synthesis of plant hormones like auxins and cytokinins, and the solubilization of phosphate and other minerals to provide nutrients (Kruasuwan and Thamchaipenet 2016; Rungin et al. 2012). Furthermore, they assist in plant growth under stressful conditions induced by drought, heavy metals, flooding and high salinity by alleviating stress associated with ethylene through the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Chen et al. 2007 correlated the role of proline accumulation with drought and salt tolerance in plants. Through these studies, we gain insight into the role of released hormones, enzymes, and other metabolic substances in alleviating salt stress conditions with the assistance of numerous rhizobacteria.

## **2. BACTERIAL MICROBES: SALT STRESS ALLEVIATION TOOL IN IMPORTANT CROPS**

Several strategies have been developed in order to decrease the toxic effects caused by high salinity on plant growth, including plant genetic engineering (W. Wang et al., 2003), and recently the use of plant growth-promoting bacteria (PGPB) (Dimkpa et al., 2009). The role of microorganisms in plant growth promotion, nutrient management and disease control is well known and well established. These beneficial microorganisms colonize the rhizosphere/endorhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (Nia et al. 2012; Ramadoss et al. 2013). Previous studies suggest that utilization of PGPB has become a promising alternative to alleviate plant stress caused by salinity (Yao et al., 2010a) and the role of microbes in the management of biotic and abiotic stresses is gaining importance.

### **2.1 CEREAL CROPS**

Cereal crops serve as the primary sources of energy and protein in the human diet, cultivated in significantly larger quantities worldwide compared to other crops. Major cereal crops include wheat, maize, rice, barley, oats, sorghum, and millet. Despite their importance, only a few of these crops exhibit salt tolerance. Traditional methods such as conventional

breeding, marker-assisted selection, and genetic engineering have been successful in enhancing yields in saline soils, but primarily for wheat and rice over the past decades. (Shahbaz & Ashraf, 2013); (Roy et al., 2014). It has been observed that the application of Salt-Tolerant Plant Growth-Promoting Rhizobacteria (ST-PGPR) in saline soil not only aids in crop survival but also enhances yields across a diverse array of cereal crops (R. P. Singh & Jha, 2016).

Similarly, Jha and Subramanian (2014) noted that the combination of *Pseudomonas pseudoalcaligenes*, an endophytic bacterium, with *Bacillus pumilus* in the rhizosphere of paddy plants was more effective in protecting the plants from abiotic stress during early growth stages. This combination induced the production of osmoprotectant and antioxidant proteins, surpassing the effects of either rhizospheric or endophytic bacteria alone. The plants inoculated with the endophytic bacterium *P.pseudoalcaligenes* exhibited notably elevated levels of glycine betaine-like quaternary compounds and increased shoot biomass, particularly evident at lower salinity levels. This study demonstrates that the detrimental effects of salinity stress can be mitigated through the application of salt-tolerant Plant Growth-Promoting Rhizobacteria (PGPR). In a glasshouse trial, this approach significantly enhanced the photosynthetic activity of all three rice varieties, resulting in higher grain yields under saline conditions (Shultana et al., 2020) (Tewari & Arora, 2014). The inoculation of endophytic *Streptomyces* sp. GMKU 336, which produces 1-aminocyclopropane-1-carboxylate deaminase (ACCD), into rice plants leads to improved growth and enhanced salt tolerance. This is accomplished by using ACCD activity to lower ethylene levels, which aids in the plants' ability to scavenge reactive oxygen species (ROS), maintain a balanced ion content, and control osmotic pressure (Jaemsaeng et al., 2018). The research conducted by (Ji et al., 2020) highlights the significant role of *Glutamicibacter* spp. YD01 in mitigating the detrimental effects of salt stress on the growth and development of rice plants. This is achieved through the regulation of phytohormone (ethylene) levels and the accumulation of reactive oxygen species (ROS), as well as maintaining ion balance, enhancing photosynthetic capacity, and promoting the expression of stress-responsive genes.

Similarly, the inoculation of endophytic *Methylobacterium oryzae* CBMB20 into salt-stressed rice plants, as observed in the study by Chatterjee et al. (2019), enhances photosynthesis and reduces emissions of stress-related volatiles. This is attributed to the modulation of ethylene-dependent responses and the activation of vacuolar H<sup>+</sup> -

ATPase. Certain strains of *Rhodopseudomonas palustris*, such as TN114, show promise in facilitating the easier and more affordable growth of rice in saline soil conditions. This is attributed to the presence of 5-aminolevulinic acid (ALA) in the examined supernatants, which has a positive effect on rice growth under such challenging conditions (Nunkaew et al., 2014). The studies by Damodaran et al. 2019 investigated the impact of various Salt-Tolerant Plant Growth-Promoting Rhizobacteria (ST-PGPR) on enhancing the productivity of salt-tolerant rice and wheat grown on sodic soils. Their findings revealed that *Lysinibacillus* sp. was particularly effective in mitigating the adverse effects of salinity. Similarly, Misra and Chauhan 2020 discovered the several *Bacillus* sp. as a ST-PGPR with ACC deaminase activity were the most dominant in alleviating salt stress and enhancing the biomass of rice across different agro-climatic zones. Furthermore, the siderophore-producing ability of microorganisms under stressful conditions presents a promising alternative to chemical fertilizers, potentially aiding in managing salt stress and iron limitations in salt-affected soils. Sultana et al. 2021, recently reported that salt-tolerant siderophore-producing PGPR supported rice growth and increase in protein content is associated with improved photosynthesis, which is indicative of higher chlorophyll levels.

Similarly, numerous studies have investigated the enhancement of saline tolerance in maize through various mechanisms employed by rhizobacteria to promote the growth and yield of the crop. The coinoculation of *Rhizobium* and *Pseudomonas* in *Zea mays* led to increased proline production, reduced electrolyte leakage, maintenance of leaf relative water content, and selective uptake of potassium ions, resulting in enhanced salt tolerance (Bano & Fatima, 2009). The Nadeem et al. 2007 discovered that inoculating salt-stressed maize with *P. syringae* (Zerrouk et al., 2016), *Enterobacter aerogenes* and *P. fluorescens* containing ACC deaminase, *Azospirillum* (Hamdia et al., 2004) resulted in higher K<sup>+</sup>/Na<sup>+</sup> ratios. This combination also led to elevated relative water content, chlorophyll levels, and reduced proline content (Vardharajula et al., 2011), indicating enhanced salt tolerance mediated by various mechanisms. The role of trehalose as an osmoprotectant under salt stress is well-documented, with numerous ST-PGPR discovered to possess genes for trehalose biosynthetic pathways (Qin et al. 2018; Orozco-Mosqueda et al. 2019; Shim et al. 2019). Aslam and Ali (2018) also reported that ACC deaminase activity in halotolerant bacterial genera such as *Arthrobacter*, *Bacillus*, *Brevibacterium*, *Gracilibacillus*, *Virgibacillus*, *Salinicoccus*, and *Pseudomonas*, as well as *Exiguobacterium* isolated from the rhizosphere and phylloplane of *Suaeda fruticosa* (L.) Forssk, stimulated the growth of maize under saline conditions.



The effect of ST-PGPR *S. sciuri* SAT-17 strain on anti-oxidative defense mechanisms and modulation of maize growth under salt stress was studied by (Akram et al., 2016). They reported that inoculation of maize with SAT-17 improved plant growth and decreased the ROS levels by increasing the cellular antioxidant enzyme activities (CAT, POD, and proline) under salinity treatments (75 and 150 mM NaCl). Likewise Upadhyay et al. 2012 studied the impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions and reported that co-inoculation with *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth with an increase in dry biomass, total soluble sugars and proline content. Nia et al. 2012 studied the effect of inoculation of *Azospirillum* strains isolated from saline or non-saline soil on yield and yield components of wheat in salinity and they observed that inoculation with the two isolates increased salinity tolerance of wheat plants; the saline-adapted isolate significantly increased shoot dry weight and grain yield under severe water salinity. Similarly, Sadeghi et al. 2012 studied the plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions and reported increases in growth and development of wheat plant. They observed significant increases in germination rate, percentage and uniformity, shoot length and dry weight compared to the control. Applying the bacterial inocula increased the concentration of N, P, Fe and Mn in wheat shoots grown in normal and saline soil and thus concluded that *Streptomyces* isolate has potential to be utilized as biofertilizers in saline soils. More recently (Ramadoss et al., 2013) studied the effect of five plant growth promoting halotolerant bacteria on wheat growth and found that inoculation of those halotolerant bacterial strains to ameliorate salt stress (80, 160 and 320 mM) in wheat seedlings produced an increase in root length of 71.7% in comparison with uninoculated positive controls. In particular, *Hallobacillus* sp. and *B. halodenitrificans* showed more than 90% increase in root elongation and 17.4% increase in dry weight when compared to uninoculated wheat seedlings at 320 mM NaCl stress indicating a significant reduction of the deleterious effects of NaCl. These results indicate that halotolerant bacteria isolated from saline environments have potential to enhance plant growth under saline stress through direct or indirect mechanisms and would be most appropriate as bioinoculants under such conditions. The isolation of indigenous microorganisms from the stress affected soils and screening on the basis of their

stress tolerance and PGP traits may be useful in the rapid selection of efficient strains that could be used as bioinoculants for stressed crops.

Similarly, Upadhyay et al. (2012) investigated co-inoculation with *B. subtilis* and *Arthrobacter* sp. could alleviate the adverse effects of soil salinity on wheat growth, leading to an increase in dry biomass, total soluble sugars, and proline content. Nia et al. (2012) they observed that inoculation with these isolates of *Azospirillum* strains isolated from saline or non-saline soil increased the salinity tolerance of wheat plants, with the saline-adapted isolate significantly enhancing shoot dry weight and grain yield under severe water salinity. The Sadeghi et al. (2012) explored the plant growth-promoting activity of an auxin and siderophore-producing isolate of *Streptomyces* under saline soil conditions, including significant improvements in germination rate, shoot length, and dry weight in wheat, also increased the concentration of nitrogen, phosphorus, iron, and manganese in wheat shoots grown in normal and saline soil, indicating the potential of the *Streptomyces* isolate as a biofertilizer in saline soils.

More recently, Ramadoss et al. (2013) investigated the effect of five halotolerant bacteria (*Hallobacillus* sp. and *B.halodenitrificans*) inoculation in wheat which mitigated salt stress in wheat seedlings, resulting in a significant increase in root length compared to uninoculated controls. These findings suggest that halotolerant bacteria isolated from saline environments have the potential to enhance plant growth under saline stress conditions through direct or indirect mechanisms. Utilizing indigenous microorganisms from stress-affected soils and screening them based on their stress tolerance and Plant Growth-Promoting (PGP) traits could expedite the selection of efficient strains for use as bioinoculants in stressed crops

## **2.2 LEGUMES AND OIL YIELDING CROPS**

Along with cereals, legumes maintain their significance as vital sources of protein in the human diet. Salinity poses challenges to the production of grain and food legumes in various regions worldwide. In legumes, salt stress negatively impacts root-nodule formation, symbiotic relationships, and ultimately, nitrogen fixation capacity (Manchanda and Garg 2008). The symbiotic association of rhizobia with legumes under salinity stress remains a

focal area of research (Zahran 1991; Zahran 1999;Graham 1992).Many studies suggest that the application of salt-tolerant rhizobia offers a sustainable solution for enhancing the productivity of legume crops grown under salinity stress (Abiala et al., 2018). Several researchers have demonstrated that the adverse effects of salinity on legumes such as soybean, pigeon pea, common bean, mung bean, groundnut, and even tree legumes can be mitigated by the application of salt-tolerant rhizobial strains (Bashan and Holguin 1997; Kumar et al. 1999; Dobbelaere et al. 2001; Bashan et al. 2004; Dardanelli et al. 2008; Meena et al. 2017;Yasin et al. 2018). The role of ACC deaminase produced by Salt-Tolerant Plant Growth-Promoting Rhizobacteria (ST-PGPR) in nodule formation in legume crops is also well-documented (Ahmad et al. 2011; Barnawal et al. 2014). During the nodulation process, ACC deaminase is crucial for enhancing the persistence of infection threads, which are negatively impacted by ethylene levels. Thus, ACC deaminase aids in nodule formation under saline conditions (Nascimento et al., 2016).

In chickpea (*Cicer arietinum* L.), delayed flowering has been directly associated with higher concentrations of Na<sup>+</sup> in the laminae of fully expanded leaves (Pushpavalli et al., 2016). The inoculation of chickpea plants with *P. putida* (MSC1) or *P.pseudoalcaligenes* (MSC4) isolates demonstrated an enhancement in various parameters such as phosphate solubilization, siderophore production, and IAA (indole-3-acetic acid) production, which are indicative of improved plant growth under salt stress conditions compared to uninoculated controls (D. Patel et al., 2012).Research by (Yilmaz & Kulaz, 2019)on chickpea highlighted the significant role of Plant Growth Promoting Rhizobacteria (PGPRs) in regulating growth under salt stress. Increased concentrations of proline, malondialdehyde (MDA), as well as enhanced activities of antioxidant enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT) were observed under saline conditions, suggesting that inoculated PGPR strains can mitigate salinity stress by enhancing salt tolerance.

A study by (Panwar, Tewari, Gulati, et al., 2016)suggested, for the first time, the potential use of native *Pantoea dispersa* strain PSB3 as a biofertilizer to mitigate the adverse effects of salt stress on chickpea plants. *P.dispersa* exhibited notable production of IAA (218.3 µg/ml), siderophores (60.33% SU), phosphate solubilization (3.64 µg/ml), and ACC (1-aminocyclopropane-1-carboxylate) deaminase activity (207.45 nmol/mg/h) even in the presence of 150 mM NaCl under laboratory conditions.The coinoculation of ACC+ *Mesorhizobium* and rhizobacterial isolates showed more stimulatory effect on nodulation and plant biomass under normal and salt amended treatments. Results revealed that positive

response of PGPR on productivity of chickpea but more enunciated response about grain yield was observed with the combined application of SA and PGPR compared to control. Growth parameters i.e root length, root mass, number of nodules and shoot mass were highly affected where SA was applied along with PGPR. From the study, it is proposed that under salt stress the combination of SA + PGPR can be a suitable practice for more production of chickpea in Pakistan.

Similarly, the coinoculation of 1-aminocyclopropane-1-carboxylate (ACC)-utilizing *Mesorhizobium* and rhizobacterial isolates demonstrated a more pronounced stimulatory effect on nodulation and plant biomass under both normal and salt-amended conditions (Chaudhary & Sindhu, 2017). The results underscored the positive impact of Plant Growth Promoting Rhizobacteria (PGPR) on chickpea productivity, with a particularly enhanced effect on grain yield observed when salicylic acid (SA) was combined with PGPR compared to control treatments. Notably, growth parameters such as root length, root mass, nodulation, and shoot mass were significantly influenced by the application of SA in conjunction with PGPR. This study suggests that the combined application of SA and PGPR could be a promising approach for enhancing chickpea production, especially in salt-stressed conditions, offering potential benefits for chickpea cultivation in (Aneela et al. 2019; Zahir et al. 2011; Zahir et al. 2010; Ahmad et al. 2011; Ahmad et al. 2013).

The enhanced mung bean growth under saline conditions, due to bacterial inoculation, might be attributed to bacterial IAA activity, which has a tremendous effect on root growth, and water and nutrient absorption from a greater soil volume (Príncipe et al., 2007). Inoculation/co-inoculation with rhizobia and plant growth promoting rhizobacteria (PGPR) containing 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase improve the plant growth by reducing the stress induced ethylene production through ACC-deaminase activity (Aamir et al., 2013). *Cronobacter* (two isolates) and *Enterobacter* (two isolates) Inoculation of PGP bacteria under 2 and 10% salinity stress showed enhanced plant growth parameters in *Vigna radiata* compared to both salinity and non-salinity control plants (Desai et al. 2023; Panwar et al. 2016b). Soil salinity poses a significant threat to plant health, impacting various aspects of their growth and development. Salinity disrupts the flowering and fruiting patterns of plants, leading to abnormalities in reproductive physiology, ultimately resulting in decreased crop yields and biomass. In the case of pigeon pea, salinity can cause a reduction in flowering by as much as 50% (*Cajanus cajan* L. Mill) (Garg & Manchanda, 2008).

## 2.3 OTHER CROPS

Salt stress profoundly impacts plant both vegetative and reproductive physiology. According to Ghanem et al. 2009, in tomato plants, exposure to salinity stress leads to the accumulation of Na<sup>+</sup> in various reproductive organs such as the style, ovaries, and anther intermediate layers. This accumulation contributes to an increase in flower abortion rates, a decrease in pollen number, and a reduction in pollen viability. Additionally, high salt stress levels, such as 150 mM NaCl, can delay flowering transition and hinder the growth of shoots and roots in tomato plants (Ghanem et al., 2009).

The Tank and Saraf 2010 demonstrated that certain Plant Growth Promoting Rhizobacteria (PGPR) capable of phosphate solubilization, phytohormone production, and siderophore secretion can enhance the growth of tomato plants under 2% NaCl stress conditions. Moreover, (Masmoudi et al., 2021) found that *Bacillus velezensis* FMH2, which produces indole-3-acetic acid (IAA), significantly promotes root length and lateral root production, thereby enhancing tomato plant growth under salt stress. These findings align with the results of studies by (Habib et al. 2016; Shultana et al. 2020) which demonstrated that PGPR inoculation increases the activities of reactive oxygen species (ROS)-scavenging antioxidant enzymes in okra and tomato plants under salt stress conditions.

Similarly Yao et al. 2010b demonstrated that inoculation with *P. putida* Rs 198 promotes cotton growth and germination even under conditions of salt stress. In Arabidopsis, the impact of salinity was investigated in a hydroponic solution, revealing various symptoms such as reduced fertility, decreased fruit length, transient wilting, and fruits predominantly containing aborted ovules and embryos, which were narrower and smaller in size (Sun et al., 2004). Similarly, salt stress affects early flowering and the male gametophyte of canola (*Brassica napus*), resulting in a reduction in pollen grain numbers and abnormal growth of anthers, ultimately leading to decreased crop yield (Mahmoodzadeh & Bemani, 2008). (Khan et al., 2012) found that under saline conditions, the growth, yield, and biomass of pearl millet are adversely affected, including reductions in germination percentage, plant height, leaf area, total biomass, and grain yield per plant. Pea plants also suffer from the adverse effects of salinity on growth, yield, and biomass (Wolde & Adamu, 2018). (Farooq et al., 2017) reviewed the impact of salt stress on grain legumes, noting that salinity can reduce crop yield by 12–100% in various legume species. (Faravani et al., 2013) investigated the salt tolerance

of black cumin (*Nigella sativa* L.) and found that increasing salinity levels from 0.3 to 9 dS m<sup>-1</sup> resulted in reduced average seed and biological yield.

Similarly, Alam et al. 2015 studied the effect of different salinity levels on the weed plant *Portulaca oleracea* L., which holds nutritional importance and is utilized similarly to spinach and lettuce in many countries. They observed reductions in biomass and yield, as well as changes in physiological attributes and alterations in stem and root structure.

### **3.SOIL REMEDIATION WITH PHYTOBENEFICIAL BACTERIA**

The industrialization of the past century has led to a significant increase in the release of anthropogenic chemicals, such as Polychlorinated biphenyls (PCBs) and persistent organic pollutants (POPs), into the environment. This has resulted in detrimental effects on human health and soil ecosystems, as highlighted by Vergani et al. 2017. Soil degradation further exacerbates these issues, stemming from factors like continuous cropping, excessive use of chemical fertilizers and pesticides, and contamination by heavy metals. Microorganisms, being the predominant biota in soil, play a crucial role in restoring land ecosystems. The microecology of the rhizosphere directly or indirectly influences the growth, development, metabolic regulation, and accumulation of active ingredients in medicinal plants (MPs). Wang et al. 2022 they suggested that, the use of microbial resources as a promising alternative to traditional fertilizers and pesticides due to their economic efficiency, environmental safety, and non-toxic nature.

The deterioration of soil quality is accelerated by emissions from industrial waste, widespread fertilizer and pesticide use, and sewage irrigation, leading to issues like soil hardening, salinization, and accumulation of heavy metals and organic contaminants (Sharma et al., 2021 Geng et al. 2019). A national survey in China revealed that 16.1% of soil sites investigated had excessive levels of pollutants, including both inorganic and organic contaminants (Mee & Mnr, 2014). Despite attempts at soil amelioration through chemical and physical methods, these approaches are often inefficient, complex, and costly (Swamy et al., 2019). Therefore, there is a growing need for more effective, economical, and environmentally friendly methods and technologies to remediate degraded soils and promote sustainable ecological and agricultural development.

Rhizoremediation has emerged as a promising strategy for in situ removal of organic contaminants, facilitated by various processes performed by different species of soil bacteria. Write your own words

### 3.1 SOIL REMEDIATION FROM SALINE STRESS THROUGH RHIZOBACTERIA

The presence of an excess amount of salt in soil shows cumulative and far-reaching effects on crops. Salt stress triggers ionic imbalance in plants, causes nutrient deficiency, perturbations in carbon (C) and nitrogen (N) assimilatory pathways, lowered rate of photosynthesis, generation of reactive oxygen species (ROS), osmotic and oxidative stress, thereby retarding growth and yield of crops (Bulgari et al. 2019; Mishra and Arora 2018).

Salt stress also poses negative impacts on soil processes, pH, decomposition rate, nutrient composition, microbial biodiversity and water availability, leading to the prevalence of drought-like conditions. According to Attia et al. 2020 in several agro-ecosystems, particularly in arid and semi-arid regions, drought and salinity occur simultaneously resulting in overlapping symptoms of both the stresses in the plants. Physical methods of treatment of saline soils, that include flushing, leaching, scraping and chemical amendments e.g. addition of gypsum and lime, are not sustainable (Egamberdieva et al., 2019). These methods are time-consuming, costly and above all, cause genetic erosion of indigenous species (Chakraborty et al. 2018; Anderson et al. 2019). Application of plant growth promoting rhizobacteria (PGPR) has the potential of alleviating salt stress in plants through elicitation of several physiological and molecular mechanisms. This includes modification in root systems, inducing antioxidant machinery, production of exopolysaccharides (useful in soil aggregate formation, humification, increase in water retention, quorum sensing, nodulation and establishing microbial diversity in saline soils) and siderophores, modulation of phytohormones, synthesis of osmolytes, uptake of minerals and control of phytopathogens (Shahzad et al., 2017)(El-Esawi et al., 2018)(N. K. Arora et al., 2018)). Several species of halotolerant soil bacteria such as *Arthrobacter*, *Azospirillum*, *Alcaligenes*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Flavobacterium*, *Pseudomonas* and *Rhizobium*, have been

reported to ameliorate salt stress in crops (Egamberdiyeva 2005; Shahzad et al. 2017; El-Esawi et al. 2018; Arora et al. 2018; Saghafi et al. 2019).

The mitigation of salt stress by halotolerant plant growth-promoting rhizobacteria (HT-PGPR) likely involves a three-tiered association: the survival of bacteria under hyperosmotic conditions, the induction of salt tolerance mechanisms in plants, and the improvement of soil quality through various mechanisms. EPS, in particular, contribute significantly to these processes by enhancing soil structure, moisture retention, and microbial interactions

### **3.2 HT-PGPR AS SOIL AMELIORATORS**

The involvement of microbial mechanisms in addressing saline soil through enhancements in structure and composition is equally significant. The presence of HT-PGPR in saline soil greatly influences soil quality and fertility parameters. Studies have confirmed that HTPGPR improve nutrient status, soil structure, organic matter, pH, EC, and deposition of ionic salts in soil (Arora and Vanza 2017; (I. Mishra & Arora, 2019)). HT-PGPR mitigate ionic toxicity through cation bridging, hydrogen bonding, and anion adsorption. There are reports where application of HT-PGPR has improved salt index of saline soil. Mitigating the nutrient status, HT-PGPR improve N, C, P, Fe and Zn content of saline soils, thereby reviving the lost vegetative index and accelerating the agricultural sustainability. Under saline conditions the N content and population of nitrogen fixers are found to be decreasing. Thus, acting as an efficient reclamation strategy, the symbiotic and asymbiotic biological nitrogen fixation by salt tolerant microbes enhances the N content as well as improves fertility of soil. The enrichment of saline soil using nitrogen fixing PGPR *Pseudomonas aeruginosa*, along with N compost stimulated the level of nitrogen as compared with un-inoculated control (Arif et al., 2017). Revival of arid and saline soil by utilizing salt-tolerant rhizobia can help in improving the fertility and productivity of these stressed agro-ecosystems (Zahran, 1999). (Hassan et al., 2018) utilized root powder of a halophyte *Cenchrus ciliaris* as carrier to develop inoculant from HT-PGPR *B. cereus*, *P. moraviensis* and *Stenotrophomonas maltophilia*. The developed bioinoculant when applied in field improved growth of wheat and simultaneously resulted in better texture, EC, pH and organic matter of saline-sodic soil. Along with N, HT-PGPR can stimulate the P, Zn and Fe content of saline soils. *P. moraviensis* reclaimed saline sodic soil by improving P, nitrate ( $\text{NO}_3^-$ ), N and K content by almost 18–35%.



Microbial mechanisms play a crucial role in addressing saline soil issues by enhancing both its structure and composition. The presence of halotolerant plant growth-promoting rhizobacteria (HT-PGPR) significantly influences soil quality and fertility parameters and various aspects of soil health, including nutrient status, soil structure, organic matter content, pH levels, electrical conductivity (EC), and the deposition of ionic salts. HT-PGPR mitigate ionic toxicity through mechanisms like cation bridging, hydrogen bonding, and anion adsorption, as indicated by research conducted by (Sandhya & Ali, 2015). Additionally, applications of HT-PGPR have been shown to enhance the salt index of saline soil, as demonstrated by (S. Arora et al., 2016).

By improving the nutrient status, HT-PGPR enhance the levels of nitrogen (N), carbon (C), phosphorus (P), iron (Fe), and zinc (Zn) in saline soils, thus revitalizing the vegetative index and promoting agricultural sustainability. In saline conditions, the nitrogen content and populations of nitrogen-fixing organisms tend to decrease. Efficient reclamation strategies involving symbiotic and asymbiotic biological nitrogen fixation by salt-tolerant microbes, as shown by (Rashid et al. 2016; Verma et al. 2019) help enhance N content and improve soil fertility.

Enriching saline soil with nitrogen-fixing PGPR, such as *P. aeruginosa*, along with nitrogen compost, as observed by (Arif et al., 2017) can stimulate nitrogen levels compared to un-inoculated controls. Innovative approaches like using root powder of halophytes as carriers for developing bioinoculants from HT-PGPR, as demonstrated by Hassan et al. (2018), show promise in improving soil conditions and crop growth. These bioinoculants have been shown to enhance wheat growth and improve soil texture, EC, pH, and organic matter content in saline-sodic soil. Furthermore, HT-PGPR can stimulate the levels of phosphorus, zinc, and iron in saline soils. For instance, *P. moraviensis* has been shown to reclaim saline sodic soil by improving P, nitrate ( $\text{NO}_3^-$ ), N, and K content by significant margins, as evidenced by (Ul Hassan & Bano, 2019)..

The increase in P content of saline soil was observed by inoculation with phosphate solubilizing *B. licheniformis* MH48 strain. Reduction in soil pH, EC and enhanced availability of macro-nutrients (NPK), micronutrients (Fe, Zn, Mn and Cu) and organic matter was reported when saline soil was inoculated with HT-PGPR and phosphogypsum (Al-Enazy et al., 2018). Besides nutrition, aggregation is also an important soil quality which promotes water percolation, root penetration, aeration and micropore formation. The

establishment of biofilm in soil aggregates or on root surface is characterized by high concentration of root exudates, signaling molecules, organic matter and water content. This complex acts as a dragging force in selecting and establishing microbial diversity. The primary content of biofilm (EPS) regulates the organic matter by serving as C source and coagulating soil particles thereby ensuring the formation of humic substances which are stable organic carbon form. Improvement of C cycling in saline soil is reported when inoculated with PGPR. Another mechanism of action reported by highlights that bacterial inoculation increases the dehydrogenase activity which is suggested to be directly correlated with soil microbial biomass which described increase in microbial biomass carbon and dehydrogenase activity in saline soil upon inoculation with HT-PGPR *B. cereus* Pb25. Research thus clearly shows the role and possible utilization of HT-PGPR in improving the quality of soils impacted with abiotic stresses such as salinity (Burns et al. 2013; Canfora et al. 2014; Lipińska et al. 2015; Islam et al. 2016).

HT-PGPR play a crucial role in enhancing soil structure and aggregation by producing extracellular polymeric substances (EPS), particularly under stressful conditions. This process leads to the formation of microaggregates, facilitating water percolation, root penetration, aeration, and micropore formation, as highlighted by Rillig et al. 2017. The establishment of biofilms in soil aggregates or on root surfaces is characterized by a high concentration of root exudates, signaling molecules, organic matter, and water content. This complex acts as a driving force in selecting and establishing microbial diversity.

Improving the productivity of saline soils holds the promise of not only bolstering food security but also enriching the content and quality of soil organic matter in these inherently nutrient-deficient agricultural systems. This endeavor offers multifaceted benefits in terms of Soil Organic Matter Enrichment, Nutrient Retention, Food Security, Soil Structure Improvement, Carbon Sequestration and achieving the sustainability.

## **FUTURE PROSPECTS**

A deep study on appropriate measure for Effective way to overcome traditional phytoremediation limitations when it comes to PCB removal, taking advantage also of the degrading ability of soil microorganisms and its efficiency in field application and a crucial issue to evaluate the concrete feasibility of this technology. Further detailed studies on “omic” approaches might act as powerful tools to unveil taxonomic and functional diversity of microorganisms, overcoming their limited cultivability, allowing the identification of the

best plant-microbe combination involved in the saline stressed soil remediation under different environmental conditions.

An additional strategy to study salt tolerant bacteria is the exploitation of resuscitation promoting factor active on those bacteria that cannot be cultivated in vitro due to their occurrence in the soil in a viable but not culturable (VBNC) state in response to stress conditions, like high pollution levels. The resurgence promoting factor (Rpf, a cytokine from the bacterium *Micrococcus luteus*) was applied on an enrichment culture established from PCB-contaminated soil, supplying biphenyl as unique carbon source. stated that the understanding of regulatory networks of ST-PGPR in inducing salt tolerance in plants, could serve as a promising measure to alleviate salt stress and improve global food productionsuggested that identification of the dominant indigenous microflora from the highly saline soil and their possible adaptation mechanisms may provide a better understanding for exploring ecological and evolutionary responses in ecosystems. The role of metagenomic and metabolomic approaches becomes very important in case of harnessing and identifying novel ST-PGPR, along with the key genes and metabolites involved in salt tolerance. especially at field scale. In particular, the potential of plants and the application of PCB-degrading endophytic bacteria remains unexplored and constitutes a big challenge due to their hydrophobic and recalcitrant chemical nature (Vergani et al., 2017) which will be a in turn benefit the plant by assimilating nutrients and increasing survival/ adaption rate (Khan et al., 2019).

An in-depth exploration into overcoming the traditional limitations of phytoremediation for PCB removal involves leveraging the degrading capacity of soil microorganisms, especially in field applications. A critical aspect to assess the feasibility of this approach is to delve into "omic" techniques, which can unravel the taxonomic and functional diversity of microorganisms, circumventing their restricted cultivability. These studies, as highlighted by (Vergani et al., 2017), aid in identifying optimal plant-microbe combinations for remediating saline-stressed soils across various environmental conditions.

A complementary strategy involves investigating salt-tolerant bacteria, utilizing resuscitation-promoting factors to revive bacteria in a viable but non-culturable state induced by stressors like high pollution levels, as elucidated by (Ma et al., 2011). Understanding the regulatory networks of salt-tolerant plant growth-promoting rhizobacteria (ST-PGPR) in enhancing plant salt tolerance, as proposed by (Kim et al., 2019), offers promising avenues to

mitigate salt stress and enhance global food production. Furthermore, uncovering the dominant indigenous microflora in highly saline soils and their adaptation mechanisms, as suggested by (Kim et al., 2019), provides insights into ecological and evolutionary responses in ecosystems. Metagenomic and metabolomic approaches play pivotal roles in identifying novel ST-PGPR, along with the key genes and metabolites involved in salt tolerance, particularly at the field scale. However, the potential of plants and PCB-degrading endophytic bacteria remains largely untapped due to the hydrophobic and recalcitrant nature of PCBs, as noted by (Vergani et al., 2017). Nonetheless, exploring this avenue presents opportunities for plants to assimilate nutrients, thereby enhancing their survival and adaptation rates under various climatic condition.

## CONCLUSIONS

The study aimed to assess the positive impacts of locally isolated salt-tolerant plant growth-promoting rhizobacteria (PGPR) on various crop growth. These enhancements likely stem from the salt tolerance and growth-promoting attributes of the chosen bacterial strains. Consequently, this promising isolate could serve as a biofertilizer source, offering potential to enhance current rice cultivation methods and address salinity challenges in coastal salt-affected regions. However, it's essential to complement this initial discovery with extensive field trials for future research, ensuring suitability for large-scale implementation. Moreover, comprehensive investigations focusing on gene expression and functional traits of salt-tolerant PGPR involved in promoting plant growth under salinity stress are imperative. These studies will facilitate the development of tailored bioformulations for saline soil systems, which are increasingly prevalent worldwide. The adoption of such green biotechnology holds multifaceted positive implications for agro-ecosystems and rural environments.

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