"Effect of Plant Growth Promoting Rhizobacteria (PGPR) on *Arachis hypogaea* under Salt Stress Condition"

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TO WHOM IT MAY CONCERN

This is to certify that **Ms. Bhawana Sethia** a student of M.Sc. Biotechnology at Nirma University, Ahmedabad, Gujarat has worked under my supervision for her M. Sc. dissertation thesis entitled **"Effect of Plant Growth Promoting Rhizobacteria (PGPR) on *Arachis hypogaea* under Salt Stress Condition"** at the Plant Omics Division of CSIR-Central Salt & Marine Chemicals Research Institute (CSIR-CSMCRI), Bhavnagar as partial fulfilment of M. Sc. Degree in Biotechnology. The thesis embodies original work done by her. Her work was found satisfactory and I wish her very good luck for her future endeavors.

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DECLARATION

I hereby declare that, to the best of my knowledge, the thesis entitled "**Effect of Plant Growth Promoting Rhizobacteria (PGPR) on *Arachis hypogaea* under salt stress condition**" submitted to the Institute of Science, Nirma University, Ahmedabad, for the partial fulfilment of the degree of Master of Science in Biotechnology (2020-2022). It was carried out under the supervision of guide **Dr. D. R. Chaudhary** (Principal Scientist, Division Plant Omics) of CSIR-CSMCRI, Bhavnagar. I assure that this data is for my practical experience and skill development and will not be published, presented, or used for any other purpose in the future. I ensure that all the data presented in this report will be treated with the utmost confidentiality.

Date: 22.04.2022

Place: CSMCRI, Bhavnagar Bhawana Sethia

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Bhawana Sethia

INDEX

ABBREVIATIONS

PGPR	Plant Growth Promoting Rhizobacteria
ROS	Reactive Oxygen Species
CAT	Catalase
SOD	Superoxide Dismutase
GPox	Guaiacol Peroxidase
GR	Glutathione Reductase
APX	Ascorbate Peroxidase
$\mu\ mg^{-1}$	Micro per milligram
$\mu\ mL^{-1}$	Micro per millilitre
$mg\ g^{-1}$	Milligram per gram
H_2O_2	Hydrogen Peroxide
EC	Electrical Conductivity
TDS	Total Dissolved Solids
pH	Potential of Hydrogen
HCN	Hydrogen Cyanide
EPS	Exo-polysaccharide
VOC	Volatile Organic Compounds
Na^+	Sodium Ions
K^+	Potassium Ions
mM	Milli Molar
ETC	Electron Transport Chain
mg	Milligram
g	Gram
mL	Millilitre
µL	Microlitre
ZMB	Zobell Marine Broth

TSA	Tryptone Soya Agar
DNS	Dinitro-Salicylic Acid
H_2SO_4	Sulfuric acid
NaOH	Sodium hydroxide
Na_2CO_3	Sodium Bicarbonate
EDTA	Ethylenediaminetetraacetic Acid
KH_2PO_4	Potassium phosphate monobasic
K_2HPO_4	Potassium phosphate dibasic
NBT	Nitroblue tetrazolium
GSSG	Glutathione disulfide
DTNB	Dithio-bis-2 nitrobenzoic acid
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
NaCl	Sodium chloride
$HgCl_2$	Mercury chloride
dS m^{-1}	Deci siemens per metre
rpm	Rotation per minute
nm	Nanometre
PVP	Polyvinyl pyrrolidone
BSA	Bovine Serum Albumin
cm	Centimetre
OD	Optical Density
ppm	Parts per million
g	Gram
µ	Micro
ppt	Parts per thousands
µS	Micro Siemens

List of Tables

List of Figures

Abstract

The applications of naturally occurring plant growth regulating microorganisms present in the halophytes plant root system can be explored to identify salt-resistant strategies and future crop advancement. The present research was designed to demonstrate the growth enhancement parameters and capabilities of salt stress-tolerant plant growth-promoting rhizobacteria (PGPR); like *Agrobacterium species*, *Klebsiella species*, *Ochrobactum species*, *Pseudomonas species*, and *Azospirillum species* in understanding the unfavorable effects of salinity stress on groundnut (*Arachis hypogaea*) seedlings. Effects of the above-mentioned halo-tolerant PGPRs on germination and development of groundnut variety Girnar-II was observed under induced salt stress up to 8 dS m^{-1}. The addition of PGPRs positively influenced the growth and germination rate of *Arachis hypogaea* seedlings, total height comprising root and shoot length, sugars, and antioxidant activity. Plantlets treated with *Pseudomonas and Agrobacterium species* showed increased plant nutrient concentrations with respected control plants. According to preliminary findings, these PGPR strains appear to be promising candidates for improving crop yield in salt-stressed agricultural systems. However, more research validation is required before acting forward with large-scale applications.

1. Introduction:

The use of high-yielding varieties, as well as chemicals, fertilisers, and pesticides, has increased agricultural output. The track to create more crop yields by using too many chemicals that cause spoilage affects arable land's chemical, physical and biological health (Pal et al., 2019). Salinity is a key environmental factor that impedes agricultural output and poses a major risk to crop production around the world (Rathore et al., 2021). High salinity has a crucial impact on plant development and physio-biochemical characteristics, leading to lower germination rate, fresh and dry weight of the plant, photosynthetic pigments, vital nutrient intake, and, most remarkably, lower crop yields (Ha-Tran et al., 2021). Plant development and yield are influenced by the availability of certain mineral elements, particularly nitrogen, phosphorous, and potassium, which are required by plants to complete their life cycle (Shaharoona et al., 2008). All cultivated crops except legumes require a relatively large amount of nitrogen (Kim & Rees, 1994). Root activity affects the rhizosphere, which has a selective influence on the associated microbial species (Poonguzhali et al., 2006). Bacteria, fungus, protozoa, and algae are only a few of the microorganisms available in the soil (Glick, 1995). Rhizobacteria are soilborne bacteria that have formed in a competitive situation to capture the rhizosphere and roots. Rhizobacteria have the ability to increase the growth of plants by two mechanisms i.e., directly by producing phytohormones, nitrogen fixation in the rhizosphere, solubilizing nutrients like Phosphorus, increasing mycorrhizal activity, and regulating ethylene production in roots (Glick, 1995; Nehl et al., 1997) by producing siderophores that chelate iron, antibiotics, and hydrogen cyanide, which inhibit the growth of fungal pathogens; and by inhibiting pathogen-produced enzymes or toxins, the indirect mechanism of PGPR reduces the negative effects of plant pathogens on crop yield (Nelson, 2004). The enhancement of crop productivity and grain quality under high salinity environments is our primary goal in plant agriculture research.

1.1. Environmental Stresses:

An environmental cause that limits crop efficiency or destroys biomass is mentioned as stress (Ashraf & Harris, 2004). Environmental stresses are categorized into two forms: Biotic and Abiotic stresses.

1.1.1. Biotic Stress:

Plants are subject to a characteristic collection of biotic stresses produced by fungi, bacteria, viruses, insects, and other living organisms. To resist biotic stress, many mechanisms have been founded. Genetic mechanisms of biotic stressors are considered to overcome these stresses in plants. By producing tolerant crops, genetically modified crops have been shown to make a significant effort against biotic stressors in plants (Gull et al., 2019).

1.1.2. Abiotic Stress:

Natural or non-living factors that affect any field's crop production are mainly caused by drought, salinity, variation in temperature whether high temperature or low temperature, nutrition damage, high light intensity, ozone (O_3), and anaerobic stress (Suzuki et al., 2014). Abiotic stress is a complex process that causes cells to make molecular, biochemical, and physiological adjustments (Shukla et al., 2012).

1.2.Salinity:

Plants deal with several biotic and abiotic stresses under severe environmental conditions. Among the various abiotic stresses, salinity is the most critical abiotic stress that reduces agricultural production. Roughly 7% of the world's land region and 20% of irrigated agricultural land are affected by salinity (Shukla et al., 2012). Salinity depends on the simultaneous recording of conductivity, temperature, and pressure (Acosta-Motos et al., 2017; Lewis & Perkin, 1978). In semi-arid and arid regions, salinity is important abiotic stress which reduces crop production (Ashraf & Harris, 2004). Due to increased salinity, about one-third of the world's fertile land suffers from poor soil quality and increased soil degradation (Rathore et al., 2021). Salt stress can cause osmotic stress and ionic toxicity, which can lead to plant death or growth dissolution, resulting in significant yield loss or even total yield failure (Xu et al., 2020). Salinity adversely affects plant growth because it reduces the amount of soil water available to plants and because of the ionic toxicity at high concentrations of specific ions that contribute to salinity (Akhtar et al., 2002). Salinity lowers plant development and growth through toxic and osmotic effects, and high values of sodium absorption rates cause acidity, increase the strength of soil, reduce root growth, and reduce hydraulic conductivity (Acosta-Motos et al., 2017).

1.3. Soil salinity:

Soil with high salt concentration affects crop yields and is known as saline soil (also called soggy soil) (Abbas et al., 2019). Soil salinity is a term that includes saline, acidic and alkaline soils defined respectively as (a) high salt concentration, (b) high sodium (Na^+) cation concentration, and (c) high pH, usually due to high concentrations of CO_3^{2-}, in the soil (Daliakopoulos et al., 2016). The halo-tolerant bacteria reduce the adverse effects of salinity through various mechanisms, including the production of phytohormones, exopolysaccharides, and phosphate solubilization (Hingole & Pathak, 2016). In the rhizosphere, the soil is said to be saline if the electrical conductivity (EC) is greater than 4 dS m^{-1} or 40 mM of NaCl. Yields of many crops decrease at this conductivity (Abbas et al., 2019). Munns concludes that the salts that plants absorb do not directly control growth but affect the photosynthesis and activity of specific enzymes (Acosta-Motos et al., 2017).

1.4. The Rhizosphere:

The thin coat of soil adjacent to the plant roots, which is extremely important and active for the activity and metabolism of the roots, is called the rhizosphere. The word rhizosphere was first coined by Hiltner. Various microorganisms are found in the rhizosphere such as bacteria, protozoa, fungi, and algae. Rhizobacteria are present at the roots of the plants and responsible for producing positive effects, ranging from direct mechanisms of action to indirect effects (Lugtenberg & Kamilova, 2009; Saharan & Nehra, 2011).

1.5. Plant Growth Promoting Rhizobacteria (PGPR):

Plant Growth Promoting Rhizobacteria (PGPR) are soil bacteria that show root colonization and help and support plants by promoting growth (Saharan & Nehra, 2011). Bacteria that are available in the rhizosphere as well as are beneficial to plants are called PGPR. These bacteria can cause plant development and growth. They participate in diverse biological activities of soil ecosystems, dynamic nutrient turnover, and long-term crop production (Gupta et al., 2015). PGPR is characterized by the following features: root surface colonization, they need to survive, grow, and fight with other microorganisms, exhibit plant growth-promoting/protective activities, and they must support plant growth. Around 2-5% of the rhizobacteria, when inoculated into soil involving competing microflora, are reintroduced into soil containing competitive microflora, which has beneficial effects on plant development and is called are plant growth-promoting rhizobacteria (Ahemad & Kibret, 2014).

1.6. Mechanism of PGPR:

Plant growth promotion occurs by modifying the overall rhizobial community in the root environment through the synthesis of different chemicals.

Plant growth is aided by PGPR either directly by their potential to supply nutrients (potassium, phosphorus, nitrogen and essential minerals) or indirectly by reducing the repressive effect of various pathogens on plant growth in the form of root killers, biological control agents and environmental protectants (Ahemad & Kibret, 2014).

1.6.1. Direct mechanism:

Rhizobacteria stimulate plant growth by using direct mechanisms like mineral nutrient solubilization, nitrogen fixation, phytohormone synthesis and organic compound mineralization, to facilitate nutrient uptake or increase nutrient availability (Arora et al., 2012; Gupta et al., 2015).

1.6.2. Indirect mechanism:

PGPR is a promising, long-term, and environmentally acceptable method of indirectly improving soil fertility and the growth of plants. This indirect method relies on a wide range of PGPR which reduces the need for fertilizers and pesticides (agrochemicals) to improve soil fertility through multiple mechanisms such as antibiotic production, stem cell, HCN production, hydrolytic enzyme production, and so on (Nelson, 2004).

1.7. Halo-tolerant PGPR:

Halotolerant PGPRs are natural microbial populations that enhance plant growth and crop yield. Halotolerant PGPRs have been related to the start of salt tolerance in several plant species, which aids in their survival and morphological development in saline environments. These salt-tolerant PGPRs support plant physiology by producing antioxidants, VOCs, EPS, and osmoregulation in plants (Abbas et al., 2019). Plant growth-promoting bacteria that are halotolerant not only improve plant development but also provide resistance to salt stress (Alexander et al., 2020).

1.8. Plant hormones produced by PGPR:

Roots secrete various bacteria in rhizomes for the roots to absorb or to control the hormonal balance of plants and to stimulate growth and respond to stress. PGPRs can induce auxins to exert effective effects on root architecture. The auxin generated by PGPR is indole-3-acetic acid (IAA), this depicts how plants and microorganisms interact. Certain PGPR strains can

support a relatively large amount of gibberellins production, leading to enhanced shoot growth. Production of cytokinin by PGPR can also lead to increased secretions from the plant's roots (Backer et al., 2018). The peak concentrations of ethylene in abiotic and biotic stresses show that it is a gaseous hormone and a stress hormone. Ethylene is necessary for plants to improve their stress tolerance to some PGPRs. Plant development is aided by bacterial plant growth promoters that include the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which significantly lowers the plant ethylene levels (Ha-Tran et al., 2021; Lugtenberg & Kamilova, 2009).

1.9. Abiotic stress tolerance associated with PGPR:

The increased potassium ion (K^+) flux to shoots and deposition of sodium ion (Na^+) on roots reduced xylem equilibrium pressure and stomatal resistance under salt stress can be eliminated by ACC deaminase production in plants, leading to increased electron transport, photosynthetic rates and ion homeostasis through deposition of Na^+ on roots, and increased K^+ flux to shoots. The increased antioxidant enzyme activity (SOD, APX, and CAT) and regulation of ROS pathway genes are all induced by PGPR (CAT, APX, GR) (Backer et al., 2018).

1.10. Reactive Oxygen Species (ROS) and Abiotic Stress:

Abiotic stresses such as drought, salinity, flooding, heat, and cold affect cell metabolism, resulting in an enhancement in reactive oxygen species production (ROS). During abiotic stress, the generation of ROS such as **$^1O_2$, $O_2^{\bullet(-)}$, H_2O_2, and HO•**, has been regarded as a consequence of stress. In plants, ROS work as both hazardous molecules and important regulators of many biological processes, including growth, cell cycle, apoptosis, hormone signaling, human responses, and biotic and abiotic development (Miller et al., 2008). Antioxidant enzymes such as superoxide dismutase (SOD), glutathione reductase (GR) guaiacol peroxidases (GPOX), catalase (CAT) and ascorbate peroxidase (APX), function together to detoxify ROS (Sarker & Oba, 2018).

1.11. Groundnut (*Arachis hypogaea L)*:

Groundnut (*Arachis hypogaea L.*) is a subtropical and tropical annual herbaceous plant belonging to the *Fabaceae* family (Yousuf et al., 2012). They are cultivated as a major source of cooking oil and vegetable protein and are essential cereal legumes. The tropical and semi-arid tropical regions produce roughly 90% of the world's groundnuts. High temperatures and low or irregular rainfall characterize these areas. For groundnut vegetative growth, a temperature range of 25-30°C is ideal. If short-term and long-term temperatures and soil

temperatures exceed optimal values, groundnut yields can be significantly reduced (Patel et al., 2013). It is a rich source of essential phytonutrients such as folate, vitamin E, antioxidants and fibres (Yousuf et al., 2012). The production of groundnut is adversely affected by a variety of abiotic and biotic stresses. The major abiotic stresses are water and salt stress. Groundnut is reasonably sensitive to salt stress. The yield of groundnut is significantly reduced (Mohan & Shashidharan, 2019). Soil salt is the primary abiotic stress that affects groundnut productivity. In semi-arid areas, salinity increases due to secondary salinization due to incorrect use of poor-quality groundwater. Salt reduces germination and seeds growth and dry matter production, and causes a deficiency of K, Fe and Ca in groundnut, resulting in a loss of yield (Singh et al., 2008).

Importance of Groundnut:

The microbial community in the groundnut rhizosphere is of great importance and can be used to develop unique bio inoculants for environmentally friendly and sustainable agricultural practices (Yousuf et al., 2012). Groundnut protein has a biological value comparable to casein and is one of the highest among vegetable proteins. Groundnut oil is well-known for its use in human nutrition as well as a cooking oil substitute. Except for B12, groundnuts are a good source of all B vitamins. It contains 1.5% P_2O_5, 7-8% N and 1.5% K_2O. It is a useful rotation crop because it enhances soil fertility by fixing atmospheric nitrogen via the root node (Yol et al., 2018).

2. Review of Literature:

2.1. Salinity:

The most important environmental stress that challenges limiting crop yields, especially in semi-arid and arid climatic regions is salinity (Acosta-Motos et al., 2017; Lewis & Perkin, 1978). Salt stress in plants, halophytes, and non -halophytes, is one of the adverse problems related to agriculture in arid regions and semi-arid (Wang & Nii, 2000). Salt stress impairs seedling growth and crop production by altering the metabolic and physiological processes (de Lima-Neto et al., 2016; B. Gupta & Huang, 2014). Salt stress leads to several changes in plants like stomatal closure and increases leaf temperature, resulting in loss of viability, and reduced seedling quality (de Lima-Neto et al., 2016; Roy et al., 2014). Plants are affected by salinity in various ways: reducing growth, reproduction, germination and vegetation, and fertility, and resulting in low seed count. Salinity causes growth inhibition by diminishing the activity of cyclins and increasing the expression of cycle-dependent kinases during the cell cycle (Abbas et al., 2019). ROS produced by salinity can cause oxidative stress to many cellular molecules such as DNA, lipids and proteins disturbing essential plant activities (Gupta & Huang, 2014).

2.1.1. Salinity effects on Soil:

Soil salinity is a result of saline irrigation, water scarcity, and sea-level rise due to global warming (de Lima-Neto et al., 2016; Gondek et al., 2020; Ha-tran et al., 2021). Soil salinity occurs in two ways: **Primary salinity** occurs naturally when soil material is the primary source of insoluble salts, whereas **secondary salinity** is caused by human activities that alter the water balance in the ecosystem, such as improper irrigation, inadequate drainage, inappropriate cropping patterns, crop rotation, chemical pollution, and vegetation cover (Abbas et al., 2019; Daliakopoulos et al., 2016). Depending on the tolerance of plants to grow in saline environments, plants are classified as halophytes or glycophytes. Glycophytes are tolerant of low salt concentrations (100–200 mM), while halophytes are tolerant of high salt concentrations (300–500 mM) (Acosta-Motos et al., 2017). Halophytes are salt-tolerant plants with physiological, morphological and molecular adaptations that enable them to thrive and complete their life cycle in high salinity. Halophytes represent roughly 1% of the world's flora. Halophytes can be used as potential crops for vegetables, oilseeds and fodder (Flowers et al., 2015).

Soil salinity has an impact on plant growth due to osmotic stress, toxic Na^+ and Cl^- ions, and imbalance in nutrition caused by excess of Na^+ and Cl^- ions (Sairam & Tyagi, 2004). Soil salinity delays or lowers plant flowering and yield. Salinity negatively effects on plant growth,

yield and flowering that have been related to (a) osmotically reduced water availability, (b) specific ion effects, and (c) plant metabolism disruption (Gill, 1979).

2.2. Mechanisms of Salt Tolerance:

The mechanisms of salinity tolerance fall into three categories:

Osmotic tolerance: Osmotic tolerance is initiated before shoot Na^+ accumulation and is regulated by signals that reduce shoot growth. The osmotic stress causes decrease in cell expansion in root tips, stomatal closure. More leaf growth would result from a reduced reaction to osmotic stress, but the increase in leaf area would benefit plants with enough soil water (Munns & Tester, 2008). Leaf area expansion is beneficial when there is a constant supply of water, but it may be unfavourable in water-stressed systems since it could reduce soil water before the ripening of the grain (Roy et al., 2014).

Tissue tolerance: High salt concentrations are observed in leaves; however, they are classified at the cellular and intracellular level, resulting in tissue tolerance (especially in the vacuole). Tolerance involves cellular and intracellular classification of Na^+ and Cl^- to prevent harmful concentrations in the cytoplasm, particularly in mesophyll cells of the leaf. After leaf Na^+ concentrations reach high levels in older leaves, toxicity develops (Munns et al., 2016).

Ion exclusion: In ion exclusion Na^+ and Cl^- transport systems in roots impedes harmful amounts of Na^+ and Cl^- from accumulating in leaves. Na^+ is kept out of the leaves by roots, ensuring it from accumulating to toxic levels in the leaves. Depending on the species, a failure in Na^+ exclusion reveals its harmful effect after days or weeks, causing the premature death of older leaves (Muchate et al., 2016; Roy et al., 2014).

2.3. PGPR:

PGPR has a number of advantageous impacts on plant growth. To be a successful PGPR, microorganisms must be able to colonize roots and establish themselves in the rhizosphere at appropriate population densities to provide beneficial effects (Kachhap et al., 2015). Induced systemic tolerance is achieved by colonizing roots with PGPRs, which improves plant development and increases secondary metabolite accumulation in the system (Tiwari et al., 2011).

2.4. Classification of PGPR:

Based on their existence in soil, biocontrol agents and plant growth promoters PGPRs can be divided into two groups:

(a) Rhizobacteria promoting extracellular bacteria (ePGPR): ePGPRs can exist in rhizomes, on rhizomes, or in the intercellular spaces in the root cortex.

(b) Rhizobacteria promoting intracellular plants (iPGPR) are usually localized within specialized nodular arrangements of root cells (Gupta et al., 2015).

2.5. Action of PGPR:

PGPRs have been distributed into two classes based on their method of action: PGPRs that indirectly stimulate plant growth and directly affect plant growth, seed development, or crop production (Podile & Kishore, 2006).

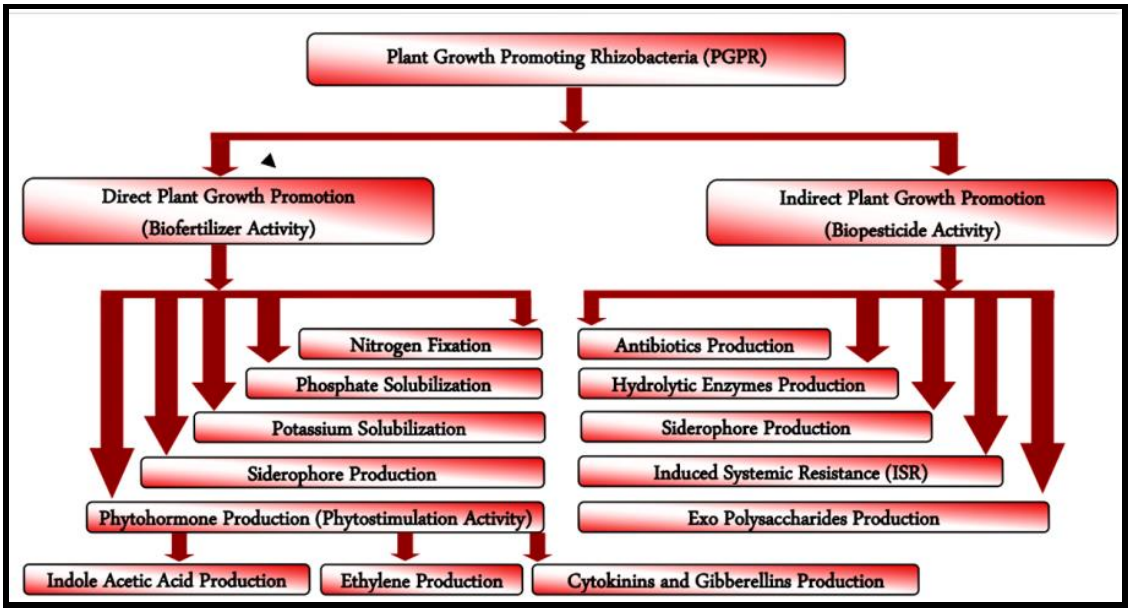

Fig. 1: - Schematic diagram showing mechanism of plant growth-promoting bacteria affect plant growth directly and indirectly (Gupta et al., 2015).

2.5.1. Direct Mechanism: Due to the deficiency of pathogens, direct PGPR promotes plant growth (Lugtenberg & Kamilova, 2009).

2.5.1.1. Nitrogen Fixation:

Biological nitrogen fixation (BNF) converts atmospheric nitrogen to plant-usable forms by converting nitrogen to ammonia by nitrogen-fixing bacteria using an enzyme, nitrogenase. Rhizobacteria that promote plant development and growth can fix atmospheric nitrogen and

transfer it to plants in two ways: Symbiotic and Non-symbiotic (Mehmood et al., 2018). The survival and dispersion of rhizobia in soil are influenced by salinity. As a result, rhizobia that successfully produce N_2-fixing rhizobium–legume symbioses under salt stress were chosen (Saharan & Nehra, 2011).

2.5.1.2. Phosphate Solubilization:

In nutritional plants phosphorus is considered as second essential growth element. In the soil, PGPR uses a variety of techniques to make use of inaccessible forms of phosphorus, thereby increasing the availability of phosphorus for plants to absorb. PGPR uses three main phosphate solubilization mechanisms: (1) Release of mineral dissolving substances, (2) Release of extracellular enzymes, and (3) Phosphate release during substrate degradation (Podile & Kishore, 2006; Walia et al., 2017)

2.5.1.3. Potassium Solubilization:

The deficiency of potassium is becoming a key restriction in crop yield as a result of unbalanced fertilizer application. With the synthesis and release of organic acids, PGPR can solubilize potassium rock. As a result, using potassium-solubilizing PGPR as a biofertilizer for agriculture development to reduce the usage of agrochemicals while also encouraging environmentally friendly crop production (Gupta et al., 2015).

2.5.1.4. Production of Phytohormones:

PGPR-produced gibberellins, ethylene, auxins and cytokinins can influence cell proliferation in the root structure by producing an overproduction of lateral roots and root hairs, resulting in increased nutrition and water intake. PGPR produces the phytohormone auxin, indole-3-acetic acid and treating plants with auxin-producing rhizobacteria improves plant development (Maheshwari et al., 2015)

2.5.1.5. Siderophore Production:

PGPR has been associated with siderophores for both indirect and direct plant growth enhancement. The direct benefits on plant growth have been established by siderophore production using radiolabelled ferric siderophores as an individual supply of iron. Siderophores, which are iron-binding ligands that attach to the ferric ion and create it accessible to the host species, are produced by organisms in order to survive. The PGPR associated with siderophores has a high chlorophyll content (Gupta, 2008).

2.5.2. Indirect Mechanism:

Plant pathogenic microorganisms pose a significant threat to global agriculture and ecosystem stability, destroying soil ecosystems, interrupting the environment, causing a drop in soil fertility with adverse effects on human's health, and contaminating groundwater (Abbas et al., 2019). Plant growth and tolerance to salinity stress are improved by indirect processes such as VOC, EPS, antioxidant defense and osmotic equilibrium (Nelson, 2004).

2.5.2.1. Antibiosis:

Bacteria release various antibiotics with different specificity and mechanisms of action in response to stressful situations (Shrestha et al., 2017). Antibiotic synthesis is a biocontrol mechanism for plant growth-promoting rhizobacteria against phytopathogens. Antibiotics have been recognized as effective in preventing the spread of plant pathogens (Podile & Kishore, 2006). One issue with relying strictly on antibiotic-producing PGPR as biocontrol agents are that some phytopathogens may acquire resistance to specific antibiotics due to the increased use of PGPR strains. Some researchers have used biocontrol strains that manufacture some antibiotics to prevent this from developing (Gupta et al., 2015).

2.5.2.2. Siderophore Production:

Siderophore-producing plant growth-promoting rhizobacteria can prevent pathogenic organisms from proliferating by isolating Fe^{3+} in the area around the root (Vejan et al., 2016).

2.5.2.3. Exopolysaccharide (EPS) Production:

EPS or biofilm produced by PGPR is critical in stimulating plant growth and development because they act as an active signal molecule during favourable interactions and provide a defence response during the infection phase (Gupta et al., 2015). The biofilm-producing PGPR enhances soil texture by increasing the number of soils macropores leading to increased water retention and nutrient supply for plants (Sáenz-Mata et al., 2016). By forming hydrophilic biofilms, the EPS-producing PGPR plays an important role in plant development during salinity (Podile & Kishore, 2006). PGPR forms biofilms on the root surface, where the cells are coated by an exopolysaccharide layer, allowing gene-regulatory systems to procreate (Abbas et al., 2019).

2.6. PGPR in Salt Tolerance:

PGPR promotes salinity tolerance by increasing root production, and biomass and increasing water efficiency (Ullah et al., 2021). PGPR increases the growth of plants such as canola, tomato, bean, lettuce, and pepper under high salinity. Some PGPR can generate cytokines and accumulate abscisic acid and antioxidants that can support ROS purification. Ethylene synthesis is important in post-transcriptional and transcriptional alterations that are regulated under salt stress in areas of the plant (Abbas et al., 2019). The unfavourable effect of ethylene is reduced by improving stress tolerance and promoting plant development. Bacteria also generate EPS, which has anti-salinity and anti-water-pressure properties and helps to improve soil structure (Timmusk et al., 2014).

2.7. Role of PGPR in alleviating drought and salinity stress in plants:

Plant stress-sensitive genes were triggered by microbial communities in recent studies, and plants grew faster, yielded more, and developed better under stressful circumstances. The different mechanism through which PGPR reduces drought and salinity stress in plants is shown in Table 1 (Abbas et al., 2019; Kaushal & Wani, 2016).

TABLE 1: Role of PGPR alleviation of drought and salinity stress in plants

S. No.	STRAINS OF PGPR	TYPE OF STRESS	CROP	COMMON NAMES	MECHANISM USED TO IMPROVED CHARACTERS OF CROP	REFERENCES
1	*Achromobacter sp*	Drought & Salinity	*Lycopersicon esculentum*	Tomato	Reduction in ethylene content	(Mayak et al., 2004)
2	*Arthrobacter sp.*	Salinity	*Pisum sativum, Triticum, aestivum L*	Pea and Wheat	Growth improvement due to an increase in nutrient uptake	(Barnawal et al., 2014; Upadhyay et al., 2012)
3	*Azospirillum sp.*	Drought & Salinity	*Helianthus Annuus L, Zea mays Arabidopsis thaliana* (Drought)	Sunflower, Maize	Enhancement in proline, ABA, chlorophyll content	(Cohen et al., 2015; Hamdia et al., 2004; Naz & Bano, 2015)

4	*Azotobacter sp.*	Salinity	*Zea mays*	Maize	Nutrition enhancement	(Rojas-Tapias et al., 2012)
5	*Bacillus sp.*	Drought & Salinity	*Vigna radiata L, Gladiolus Zea mays* (Drought)	Mung bean Maize	Improve the activity of ACC deaminase. The activity of SOD, CAT, phenols, increases Increased proline, K^+ uptake. APX, CAT, and GPox activity reduced in maize	(Damodaran et al., 2014; Li & Jiang, 2017; Patel et al., 2015; Vardharajula et al., 2010)
6	*Burkholdera sp.*	Salinity	*Cucumis sativus*	Cucumber	Increase in water and chlorophyll levels	(Kang et al., 2014)
7	*Haereohalobacter sp.*	Salinity	*Arachis hypogaea L.*	Groundnut	K^+ content improved	(Shukla et al., 2012a)
8	*Halo bacillus sp.*	Salinity	*Sesuvium portulacastrum*		HCN and ammonia production	(Desale et al., 2014)
9	*Klebsiella sp.*	Salinity	*Triticum aestivum, Avena sativa*	Wheat Oat	Increase in K^+ and proline level	(Sapre et al., 2018; R. P. Singh et al., 2015)
10	*Ochrobactrum sp.*	Salinity	*Arachis hypogaea L*	Groundnut	IAA and ACC deaminase production	(Paulucci et al., 2015)
11	*Pantoea sp.*	Drought & Salinity	*Vigna radiata L.* *Triticum, aestivum L* (Drought)	Mung Bean Wheat	ACC deaminase activity enhanced the salt tolerance EPS formation enhances the drought tolerance	(Amellal et al., 1998; Panwar et al., 2016)

12	*Pseudomonas sp.*	Salinity	*Lycopersicon esculentum Arachis hypogaea*	Tomato Groundnut	Improvement in plant growth due to an increase in proline, IAA, and EPS production	(Ali et al., 2014; Saravana kumar & Samiyappan, 2007a)
13	*Rhizobium sp.*	Drought & Salinity	*Pisum sativum, Zea mays Helianthus Annuus L* (Drought)	Pea Maize Sunflower	Increase in chlorophyll and photosynthesis rate for salt tolerance Drought tolerance by EPS formation in sunflower	(Alami et al., 2000; Bano & Fatima, 2009)
14	*Serratia sp.*	Salinity	*Triticum aestivum L*	Wheat	Salt tolerance by EPS formation	(R. P. Singh & Jha, 2016)
15	*Streptomyces sp.*	Salinity	*Limonium sinense, Solanum lycopersicum,*	Girard Tomato	Proline production	(Palaniyandi et al., 2014; Qin et al., 2017)

2.8. Applications of PGPR:

Growth Enhancement:

Plant growth is improved by PGPRs due to various factors, including the release of plant hormones, regulation of ethylene production in the roots, nitrogen fixation, solubilization of nutrients, production of accessory cells, and promoting of root function and reducing heavy metal toxicity are the most important (Prasad et al., 2015). Plant growth-promoting rhizobacteria (PGPR) can improve germination, seedling emergence, and growth (Parewa et al., 2014).

Maintenance of Soil Fertility and Nutrient uptake:

PGPRs alter the nutritional, physical and physiological properties of rhizospheric soils and indirectly influence the patterns of soil microbial life in that area. Rhizobacteria increase the plant's ability to absorb nutrients such as Potassium, Calcium, Iron, Copper and Zinc through stimulation of the proton pump ATPase (Bhattacharyya & Jha, 2012).

PGPR as Biofertilizers:

Bio-fertilizers are substances made from living microorganisms that can penetrate the rhizome of the plant when applied to seeds or the plant surface adjacent to the soil, promoting root growth (Bhattacharyya & Jha, 2012). PGPR acts as biofertilizers, either directly by supplying nutrients to the host plant or indirectly by influencing root growth and structure or supporting other beneficial symbiotic connections (Vessey, 2003).

PGPR as Biocontrol Agents:

PGPRs have the ability to resist plant diseases, referred to as biological control. PGPR as the biochemical agent has a few benefits over chemical control agents. PGPR is helpful, naturally occurring microorganisms that are harmless and environmentally favorable (Labuschagne et al., 2010). *Pseudomonas* is the best identified biological control agent at the molecular level. They can proliferate in vitro, rapid utilization of root and seed secretions, colonize and multiply in the environment of the rhizosphere within plants, and intense competition with other microorganisms(Saharan & Nehra, 2011).

2.9. Effects of Stress on Compatible Solutes:

The four basic classes of compatible solutes are carbohydrates, polyols, amino acids, and quaternary ammonium compounds. Salinity leads to an increase in compatible solutes (Puniran-Hartley et al., 2014). Proline deposition acts as an osmotic protectant in response to salinity stress, and higher levels indicate greater salt and drought tolerance (Ranganayakulu, G. S et al., 2013). Salt stress causes carbohydrate levels to rise, such as sugars and starch. These carbohydrates' main functions in stress reduction are osmoprotectant, carbon storage, and reactive oxygen species scavenging. Salt stress causes cells to manufacture more reducing sugars, according to research (Gupta & Huang, 2014).). Salinity and drought stress is caused by the accumulation of soluble sugars in plants, which act as osmolytes (Ranganayakulu, G. S et al., 2013).

2.10. Reactive Oxygen Species (ROS):

Vehicle exhaust, tobacco smoke, ozone, and low-wavelength electromagnetic and ultraviolet (UV) radiation are the key causes of ROS. Autoxidation of amino acids, phagocyte respiratory bursts, ischemic reperfusion damage, and the mitochondrial ETC are all producers of endogenous ROS (Haida & Hakiman, 2019). ROS serves as an indicator molecule for signaling pathways that induce adaptive/protective responses, with H_2O_2 serving as a secondary messenger (Sarker & Oba, 2018). Salinity stress has been known to increase the manufacture of reactive oxygen species and damage caused by ROS. ROS produces oxidative

damage to cells in reaction to stress. In plants, reactive oxygen species (ROS) are crucial signaling molecules that mediate responses to pathogen infection and stresses (Miller et al., 2010). Some of the significant reactive oxygen species-scavenging enzymes present in plants comprise catalase, superoxide dismutase, ascorbate peroxidase, glutathione peroxidase, and peroxiredoxin. They provide cells with highly capable detoxification machinery for O_2^- and H_2O_2 (Mittler et al., 2004).

2.11. Future Aspects:

Inoculating salt-tolerant PGPR into the plant cell suspension and regenerating embryos and producing stress-tolerant plants is an opportunity for effective inoculation, which relates to plants created from tissue culture (Arora et al., 2012). The application of different strains of PGPR will inspire more research to improve the rhizosphere and provide new potential for sustainable agriculture and nutrient management (Ansari et al., 2017). More research into selecting appropriate rhizosphere microbes and forming microbial communities, as well as multidisciplinary research combining applications in biotechnology, nanotechnology, agrobiotechnology, chemical engineering, and material science, as well as combining different ecological and functional biological approaches, could lead to new preparations and opportunities with enormous potential (Prasad et al., 2019). Future research into improving growth conditions, broad-spectrum action, safety, stability, and shelf life of PGPR products is critical to the commercialization success of PGPR (Mhatre et al., 2019).

Objectives of the study:

1. To observe the growth and development of PGPR treated groundnut seeds under salt concentrations.
2. To study various osmolytes such as sugar concentration in PGPR treated groundnut plants under salt stress.
3. To analyze the antioxidative enzyme activity of groundnut plant samples towards salt stress.

3. Materials and Methods:

3.1. Materials:

3.1.1. Source of Halotolerant bacteria (PGPRs):

The halo-tolerant bacteria or the microbial strains which were used here for analysis were already isolated and characterized at the CSIR-CSMCRI laboratory and were stored at -80° C in glycerol stock. Few of these microbial strains were here used for this study. Eight microbial strains are used here further mentioned in this study. The codes given to these eight bacterial strains as Microbial Strain-1 (MS-1) and so on. Microbial strains used here are as follows in table 2.

Table 2: Bacterial strains used with given code and species name.

S.No.	Code	Species Name
1	MS-1	*Agrobacterium species*
2	MS-2	*Klebsiella species I*
3	MS-3	*Ochrobactrum species*
4	MS-4	*Pseudomonas species I*
5	MS-5	*Pseudomonas species II*
6	MS-6	*Klebsiella species II*
7	MS-7	*Klebsiella species III*
8	MS-8	*Azospirillum species*

3.1.2. Groundnut Seed sample collection:

Groundnut (*Arachis hypogaea*) seeds were collected from the Indian Council of Agricultural Research-Directorate of Groundnut Research (ICAR-DGR), Junagadh, Gujarat. The species in focus the was Girnar-II groundnut species.

3.1.3. Composition of Growth media used:

Zobell Marine Broth (ZMB): 40.25g ZMB dissolved in 1000 mL Milli-Q water. The composition of ZMB media is given in the appendix.

Tryptone Soya Agar (TSA): 40g TSA dissolved in 1000 mL Milli-Q water. The composition of TSA media is given in the appendix.

3.1.4. Reagents Preparation:

3.1.4.1. Biochemical Analysis:

For biochemical analysis, the extraction of plant samples stored at -80° C were crushed in liquid N_2 with help of 80% Ethanol.

80% ethanol: 800 mL ethanol dissolved in 200 mL Milli-Q water (1000 mL volume).

Starch:

52% Perchloric Acid –222.85 mL of perchloric acid and distilled water were added to make up the volume to 300mL.
Standard Glucose Solution: 1% Stock – 10mg glucose/10mL distilled water.
Working Solution – 1mL 1% Stock glucose solution dissolved in 9mL distilled water.
Anthrone Reagent: 24mL distilled water was added to 456mL H_2SO_4 to make 95% H_2SO_4 and to this 960mg of anthrone was added.

Soluble sugar:

Standard Glucose Solution: 1% Stock – 10 mg 10 mL^{-1} distilled water.
Working Solution – 1 mL glucose solution dissolved in 9 mL distilled water.
Anthrone Reagent: 24 mL distilled water was added to 456 mL H_2SO_4 to make 95% H_2SO_4 and to this 960 mg of anthrone was added.

Reducing sugar:

Standard Glucose Solution: 1% Stock – 10 mg 10 mL^{-1} distilled water. Dinitro-Salicylic Acid (DNS): 1.06 g DNS and 1.98 g NaOH were dissolved in 141.6 mL Milli-Q water. The yellow color was observed on mixing. In this solution, 30.6 g Na-K tartrate, 760 µL phenol, and 850 mg Na-meta-sulphate were dissolved.

Polyphenols:

20% Na_2CO_3 solution: 50 g Na_2CO_3 dissolved in 250 mL distilled water on a magnetic stirrer.

Standard catechol solution (1% stock): 10 mg catechol dissolved in 10 mL distilled water.
Working catechol solution: 1 mL stock dissolved in 9 mL distilled water.

3.1.4.2. Antioxidant activity Analysis:

Extraction Buffer preparation:

(i) 1mM EDTA – 3.72 g EDTA dissolved in 100 mL phosphate buffer

(ii) TritonX100 solution: 100 µL tritonX100 solution dissolved in 20 mL phosphate buffer.

(iii) 50mL phosphate buffer (pH=7.0)

Catalase:

Phosphate buffer (50mM): pH adjusted to 7.0

(i) Potassium phosphate monobasic (KH_2PO_4): 1.701 g KH_2PO_4 dissolved in 250 mL distilled water.

(ii) Potassium phosphate dibasic (K_2HPO_4): 2.177 g K_2HPO_4 dissolved in 250 mL distilled water.

Hydrogen Peroxide (H_2O_2) (30mM): 0.34 mL (340 µL) H_2O_2 dissolved in 100 mL phosphate buffer.

Superoxide dismutase (SOD):

Phosphate buffer (50mM): pH adjusted to 7.8

(i) Potassium phosphate monobasic (KH_2PO_4): 1.701 g KH_2PO_4 dissolved in 250 mL distilled water.

(ii) Potassium phosphate dibasic (K_2HPO_4): 2.177 g K_2HPO_4 dissolved in 250 mL distilled water.

L-Methionine: 2.215 g L-Methionine dissolved in 50 mL phosphate buffer.

Nitroblue tetrazolium (NBT): 142.27 mg NBT dissolved in 100 mL phosphate buffer.

Riboflavin: 1.355 mg riboflavin dissolved in 50 mL phosphate buffer.

TritonX100 solution: 500 µL solution dissolved in 50 mL phosphate buffer.

Guaiacol peroxidase (GPox):

Phosphate buffer (50mM): pH adjusted to 7.0

(i) Potassium phosphate monobasic (KH_2PO_4): 1.701 g KH_2PO_4 dissolved in 250 mL distilled water.

(ii) Potassium phosphate dibasic (K_2HPO_4): 2.177 g K_2HPO_4 dissolved in 250 mL distilled water.

Guaiacol: 627.35 µL guaiacol dissolved in 50 mL phosphate buffer.

Hydrogen Peroxide (H_2O_2) (57mM): 646 µL H_2O_2 dissolved in 100 mL phosphate buffer.

Glutathione reductase (GR):

Phosphate buffer (100mM): pH adjusted to 7.5

(i) Potassium phosphate monobasic (KH_2PO_4): 3.402 g KH_2PO_4 dissolved in 250 mL distilled water.

(ii) Potassium phosphate dibasic (K_2HPO_4): 4.354 g K_2HPO_4 dissolved in 250 mL distilled water.

EDTA (30mM): 131.508 mg EDTA dissolved in 15 mL phosphate buffer.

GSSG oxidized: 275.67 mg GSSG dissolved in 15 mL phosphate buffer.

DTNB (Ellman's reagent): 133.77 mg DTNB dissolved in 15 mL phosphate buffer.

NADPH (3mM): 33.495 mg NADPH dissolved in 15 mL phosphate buffer.

3.1.4.3. Diacid Solution:

Diacid solution is comprised of Nitric Acid (HNO_3): Perchloric Acid ($HClO_4$) in the ratio of 10:4 for which 750 mL nitric acid was mixed with 300 mL perchloric acid.

3.2. Methods:

3.2.1. Bacterial culture preparation:

For bacterial inoculum preparation, ZMB media tubes containing 10 mL media were prepared, and a single colony from each strain (MS-1 to MS-8) was inoculated in respective tubes and kept on overnight incubation at 30° C and 120 rpm in a mechanical shaker. A fresh 100 mL ZMB media was prepared in which 80 mM (8 dS m^{-1}) NaCl water was used for media preparation instead of distilled water and 100 µL overnight grown culture was added to each flask separately and kept in overnight incubation for culture growth. Next morning cell culture growth was measured using a spectrophotometer and cultures are ready to use for seed treatment.

3.2.2. Groundnut seed surface sterilization and PGPR treatment:

The Girnar-II variety groundnut seeds were surface sterilized by the conventional method. Seeds were first washed with 70% ethanol and then rinsed with 0.1% $HgCl_2$ solution for 5 to 10 minutes. At last, groundnut seeds were given three washes of running tap water.

These surface-sterilized seeds were now used for bacterial treatment. As per our eight microbial strains, groundnut seeds were dipped in fully grown cultures for 2 hours and then placed on blotting paper in petri plates. All this seeds treatment work is carried out in a laminar airflow chamber. These petri plates were put in culture room condition for the first 24 hours and then at room temperature for better germination. These fully germinated seeds were further used outdoor, sowing in pots at the greenhouse.

3.2.3. Outdoor seed germination:

The ex-vitro groundnut cultivation and germination work were carried out at the greenhouse centre at CSIR-CSMCRI, Bhavnagar location. The local Gujarat field soil was used for pot germination. The soil was taken under for basic characterization analysis. The different physicochemical parameters of the soil sample, including total salinity, electrical conductivity (EC), total dissolved solids (TDS), pH, etc. were determined using an EC meter and pH meter. Approximately 3.5 kg of soil was filled in every pot and watered initially 2 times.

The in-vitro 2-3 days germinated seeds were then used for sowing in the above pots according to two different treatments with one control and the other eight PGPR treated seeds under salt stress. Each treatment has 6 replicates for better data presentation. Within 6 days all the seeds were successfully grown in pot soil. Initially, for 15 days normal tap water was given to the plants and were maintained under shade conditions. After the establishment of small plantlets, we started giving salt water in increasing order every two-day interval time. At last, 8 dS m^{-1} NaCl water was given to plantlets till their harvesting.

3.2.4. Sample harvesting and preservation:

Groundnut plant samples were taken for harvesting after 60 days of sowing and 35 days after salinity was given. Plant leaf samples were collected in duplicates and stored at -80° C for the analysis of osmolytes estimation and antioxidant enzyme activity check under salt stress conditions. Then the whole plant was uprooted from the base and brought to lab condition.

3.2.5. Morpho-physiological analysis:

After completion of the pot experiment, groundnut plant samples were harvested. Root and shoot length (Total Height) were measured for all the plant samples using a measuring scale. The freshly harvested plants were weighed by using an electrical balance to estimate the plant's fresh weight. Plants were then oven dried at 60° C for 48 hours and again weighed on electrical balance to estimate the dry weight of the plant.

3.2.6. Biochemical attributes analysis:

Ethanolic extraction of the plant samples was done. For extraction, 300 mg of fresh plant samples were crushed with liquid nitrogen and collected in eppendorf and 4 mL 80% ethanol was added. The eppendorfs were centrifuged and pellets were extracted 2 times by centrifugation. After centrifugation, the supernatants were collected and kept for the evaporation of ethanol at 50 ºC in a hot air oven, and to the obtained residues 4 mL of Milli-Q water was added, and from this further estimation of soluble sugars, reducing sugars, total amino acids, and polyphenols was done. The pellets were used for starch estimation (Rathore et al., 2021).

3.2.6.1. Starch analysis:

Estimation of starch was done by Hansen and Moller method in which anthrone reagent was used (Hansen & Møller, 1975). The pellets collected from alcoholic extraction were digested in 52% perchloric acid and then centrifuged at 7200 rpm for 10 minutes at room temperature. After centrifugation 20 µL supernatant was collected and the volume was made up to 1mL (i.e., 980 µL) with Milli-Q in tubes. 4 mL anthrone reagent was added to this sample solution. The tubes were incubated at 90ºC for 15 minutes in a water bath and then cooled on ice or at room temperature for some time. Optical density was observed at 630 nm on a UV-Vis spectrophotometer (EPOCH UV-Vis spectrophotometer) in 96 well plate. A standard solution of glucose at the concentration of 10 to 100 µg mL^{-1} was prepared and used to prepare the standard curve.

3.2.6.2. Soluble sugar analysis:

The anthrone reagent method was used in the estimation of soluble sugars as described by Hansen and Moller in 1975 (Hansen & Møller, 1975). 200 µL extract (residue+ Milli-Q water) was taken in the tube and the volume was made up to 1 mL (800 µL) with Milli-Q in tubes. 4 mL anthrone reagent was added to the sample solution. The tubes were incubated at 90º C for 15 minutes in a water bath and then cooled down on ice or at room temperature. Absorbance was observed at 630 nm on a spectrophotometer (EPOCH UV-Vis spectrophotometer) in 96 well plate. A standard solution of glucose at a concentration ranging from 10 to 100 µg mL^{-1} was prepared and used to make the standard curve.

3.2.6.3. Reducing sugar analysis:

Estimation of reducing sugars was done by using DNS reagent according to Miller's method (G. L. Miller, 1959). 500 µL sample extract (residue+ Milli-Q water) was taken in the glass tube and the volume was made up to 2 mL (i.e., 1500 µL) with Milli-Q water in tubes. 1 mL DNS reagent was added to the reaction mixture. The tubes were incubated in a water bath for 10 minutes at 90º C and after incubation orange-red color was observed and tubes were cooled on ice or at room temperature. 100 µL samples were placed on 96 well plate and optical density was observed at 540 nm on a spectrophotometer (EPOCH UV-Vis spectrophotometer). Glucose at the concentration of 100 to 1000 µg mL^{-1} was prepared was used to prepare the standard curve.

3.2.6.4. Polyphenol analysis:

Polyphenols in the plant samples were estimated by the method given by Chandler and Dodds (1983) (Chandler & Dodds, 1983). 200 µL of extract (i.e., residue+ Milli-Q water) was taken in the tube and the volume was made up to 2 mL with Milli-Q (1800 µL) in tubes. 0.5 mL Folin-Ciocalteau's reagent and 0.5 mL Milli-Q water were added to the reaction mixture and mixed well. 2 mL 20% Na_2CO_3 solution was added to the reaction mixture. The tubes were incubated at 90º C for 1-2 minutes in a water bath and dark bluish color was observed in the tubes then the tubes were cooled on ice or at room temperature. Optical density was observed at 650 nm on a spectrophotometer (EPOCH UV-Vis Spectrophotometer) on a 96-well plate. Catechol at the concentration range of 10 to 100 µg mL^{-1} was used to prepare the standard curve.

3.2.7. Antioxidant enzyme activity analysis:

Frozen plant samples (300 mg) were crushed with the help of liquid N_2 and protein was extracted with 2 ml of 50 mM 50mL potassium phosphate buffer (pH 7.0) containing 1 mM EDTA (500 µL), 0.5% TritonX-100 solution (5 mL), and a small amount of 5% polyvinyl pyrrolidone (PVP). The samples were centrifuged at 13,000 rpm for 20 min at 4°C. The supernatant was collected in an eppendorf after centrifugation, the final samples were called protein extracts and were used for antioxidant enzyme activity (Sarker & Oba, 2018).

Protein estimation of these extracted substances was done by following the Bradford method (1976) in which Bovine Serum Albumin (BSA) was kept as standard (Bradford, 1976).

3.2.7.1. Catalase (CAT) activity:

Aebi's method was used for the analysis of catalase activity in the plant samples (Aebi, 1984). 3 mL of reaction mixture containing 1900 µL 50 mM of potassium phosphate buffer (pH 7.0) and 100 µl of enzyme extract and the reaction was initiated by adding 1 mL 30 mM of hydrogen peroxide. The optical density was measured by using a spectrophotometer (Carry 500 UV-Vis Spectrophotometer) at 240 nm for 3 minutes (7 cycles of 30 sec each). The catalase activity was calculated by using the following formula:

$$\textbf{Enzyme Activity} = \frac{\mathbf{V}}{\boldsymbol{\varepsilon} * \mathbf{d} * \mathbf{v} * \mathbf{Cp}} * \frac{\Delta \mathbf{E}}{\Delta \mathbf{t}}$$

where,

V= Assay volume/ volume of sample in the cuvette

ε= Extinction coefficient = $43.6*10^{-3}$ mM^{-1} cm^{-1}

d= Light path=1cm

v= volume of extract

Cp= concentration of protein

ΔE= change in OD

Δt= time interval

3.2.7.2. Superoxide dismutase (SOD) activity:

SOD estimation was done by Misra & Fridovich's (1972) method with some slight modifications (Misra & Fridovich, 1972). The final volume of the reaction mixture was 3 ml which contains 2525 µL of 50 mM of potassium phosphate buffer (pH 7.8), 100 µL of L-

methionine, 75 µL of triton X-100 solution, 100 µL of Nitroblue tetrazolium (NBT), 100 µL of riboflavin and 100 µL extract. the reaction mixture containing tubes was kept in light for 20 minutes for incubation. 1 tube as blank and 1 as control (both containing 100 µL of phosphate buffer instead of extract) were used and the control was kept in dark for 20 minutes for incubation. The absorbance was measured by spectrophotometer (EPOCH UV-Vis Spectrophotometer) at 560 nm in a 96-well plate. The SOD activity was calculated by the given formula:

$$\textbf{\% Inhibition} = \frac{ODcontrol - ODsample}{ODcontrol - ODblank} * 100$$

$$\textbf{Enzyme Activity} = \frac{\%\, Inhibition * \Delta F}{50 * Cp}$$

where, Cp = concentration of protein

3.2.7.3. Glutathione peroxidase (GPox) activity:

The Guaiacol oxidation method illustrated by Britton and Mehley (1955) was used for the estimation of glutathione peroxidase activity (Chance et al., 1955). In the 3 mL cuvette, final reaction mixture was added which consists of 1450 µL of potassium phosphate buffer (pH 7.0), 540 µL of guaiacol, 10 µL of enzyme extract, and 1000 µL of H_2O_2 was added to initiate the reaction. Optical density was measured by a spectrophotometer (Carry 500 UV-Vis Spectrophotometer) at 470 nm. Enzyme activity shown by the plant samples is calculated by the given formula here:

$$\textbf{Enzyme Activity} = \frac{v}{\varepsilon * d * v * Cp} * \frac{\Delta E}{\Delta t}$$

where,

V= Assay volume/ volume of sample in the cuvette

ε= Extinction coefficient = 26.6 mM^{-1} cm^{-1}

d= Light path=1cm

v= volume of extract

Cp= concentration of protein

ΔE= change in OD

Δt= time interval

3.2.7.4. Glutathione reductase (GR) activity:

Glutathione reductase estimation was carried out by the method represented by Connell and Mullet with minor modifications (Connell & Mullet, 1986). In the 3 mL cuvette final reaction mixture (3ml) was taken which was composed of 100 µl enzyme extract, 2500 µL phosphate buffer (pH 7.5), 100 µL GSSG, and 100 µL DTNB and at last, 100 µL NADPH was added in the reaction mixture. The absorbance was measured by using a spectrophotometer (Carry 500 UV-Vis Spectrophotometer) at 412 nm. GR activity was calculated by the below formula:

$$\textbf{Enzyme Activity} = \frac{\mathbf{v}}{\boldsymbol{\varepsilon} * \mathbf{d} * \mathbf{v} * \mathbf{Cp}} * \frac{\boldsymbol{\Delta}\mathbf{E}}{\boldsymbol{\Delta}\mathbf{t}}$$

where,

V= Assay volume/ volume of sample in the cuvette

ε= Extinction coefficient = 26.6 mM^{-1} cm^{-1}

d= Light path=1cm

v= volume of extract

Cp= concentration of protein

ΔE= change in OD

Δt= time interval

3.2.8. Plant Nutrient Analysis:

The groundnut plant samples which were dried in a hot air oven were crushed into a fine powder with help of a Retsch Mixer Mill (MM-400) (homogenizer) and this fine powder of groundnut plant samples is collected and used for elemental analysis. Major essential plant nutrient elements like Nitrogen (N), Carbon (C), Sulphur (S), Phosphorus (P), Sodium (Na), Potassium (K) and other micro-elements were analyzed. C, N, and S content of fine groundnut plant samples were analyzed by Vario Mario Cube Elemental Analyzer.

Acid Digestion:

Di-acid digestion for crushed groundnut plant samples were carried out for analysis of remaining micronutrients. For acid digestion, the groundnut plant samples were digested by diacid composed of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a ratio of 10:4. 100 mg plant samples were taken in 100 mL conical flask and 10 mL diacid was added to it. Then these flasks were heated on a magnetic stirrer at 400° C temperature till the acid evaporated. The remaining less than 1 mL content was taken and 25 ml distilled water was added. Then this was

filtered through Whatman paper and collected for analysis. This filtrate was further used for other micronutrients estimation and sodium-potassium analysis.

3.2.8.1. Sodium and Potassium analysis:

The acid digested samples were used here again for estimation of sodium and potassium concentration of groundnut plant samples. A flame photometer is used here for determining the ion content in ppm for sodium and potassium. First, the flame photometer was calibrated with standard sodium and potassium solution with a range of 10 ppm to 100 ppm concentration.

4. Result and Discussion:

4.1. Physicochemical characters of the soil used:

Soil physicochemical factors such as electrical conductivity (EC), pH, salinity, and total dissolved solids (TDS) were measured before seeds were planted. The result is shown in table 3.

Table 3: Physiological characters of soil:

PHYSICOCHEMICAL PARAMETERS	OBSERVATION
Electrical Conductivity (EC)	298.06 µS cm^{-1}
Total Dissolved Solids (TDS)	193.16 mg L^{-1}
pH	8.91
Salinity	0.14 ppt

4.2. Morpho-physiological analysis:

Growth parameter analysis of groundnut plants grown under salt stress includes total height (root length + Shoot length), fresh weight and dry weight. The enhancement in PGPR-treated plants were observed as compared to the control. The root length of groundnut plants was reduced by salt stress, and this response was more prominent in PGPR-treated plants. The minimum total height was of the control plant (41.67 cm) and the maximum height was observed in *Azospirillum* strain (65.83 cm). All the bacterial treated plants have more height in comparison to control plant. Also, in case of fresh and dry weight comparison between control and PGPR treated plants, the *Azospirillum* strain showed the highest values among all other PGPR treated plants. Fresh weight (5.44 gm) and dry weight (1.16 gm) given by *Azospirillum* species which is much better than the control plant (1.80 gm F.W. and 0.70 gm D.W.) (Table-4).

Figure 2 shows the height of groundnut plants under salt stress. In comparison to the control, the number of leaves increased in all bacterial strains treated plants.

Fig. 2: Growth of PGPR-treated groundnut under salinity conditions (A) Control (B) MS-1 (C) MS-2 (D) MS-3 (E) MS-4 (F) MS-5 (G) MS-6 (H) MS-7 (I) MS-8.

Table 4: Morpho-physiological parameters of plant with control eight MS treated plants.

	Total Height (cm)	Fresh Weight (g)	Dry Weight (g)
Control	41.67	1.80	0.70
MS-1	47.83	2.94	0.90
MS-2	54.33	3.36	0.88
MS-3	59.00	4.21	1.16
MS-4	44.33	3.33	0.91
MS-5	56.00	3.67	0.96
MS-6	55.83	3.45	0.96
MS-7	58.17	4.06	1.07
MS-8	65.83	5.44	1.16

The increase in height of plants can be due to the association between groundnut plants and rhizobia. The biological nitrogen-fixing bacteria is important for the growth and development of groundnut plants. Previous research has shown that *Azospirillum sp.* improves the growth by increasing the dry weight of both the root system and aerial parts of PGPR inoculated plants (Bhattacharyya & Jha, 2012).

The increased production of plant growth regulators and mobilization of available nutrients by PGPRs are responsible for the increased growth. Nitrogen fixation can also aid plant development (Sharma et al., 2016). Previous studies showed that plant growth and development are inhibited due to salinity through ion toxicity and imbalance and by exerting low water potential (Mensah et al., 1973).

4.3. Biochemical Analysis:

4.3.1. Starch analysis:

The total starch content of all the MS's treated groundnut plants ranges from 27.2 mg g^{-1} to 99.2 mg g^{-1} whereas the control is having only 28.9 mg g^{-1} except *Agrobacterium sp.* (MS-1) which has starch concentration of 27.2 mg g^{-1}, there is a slight decrease in starch content compared to control plants. The maximum concentration of starch is observed in *Ochrobactrum sp.* (MS-3) treated plants i.e., 99.2 mg g^{-1} (Figure 3).

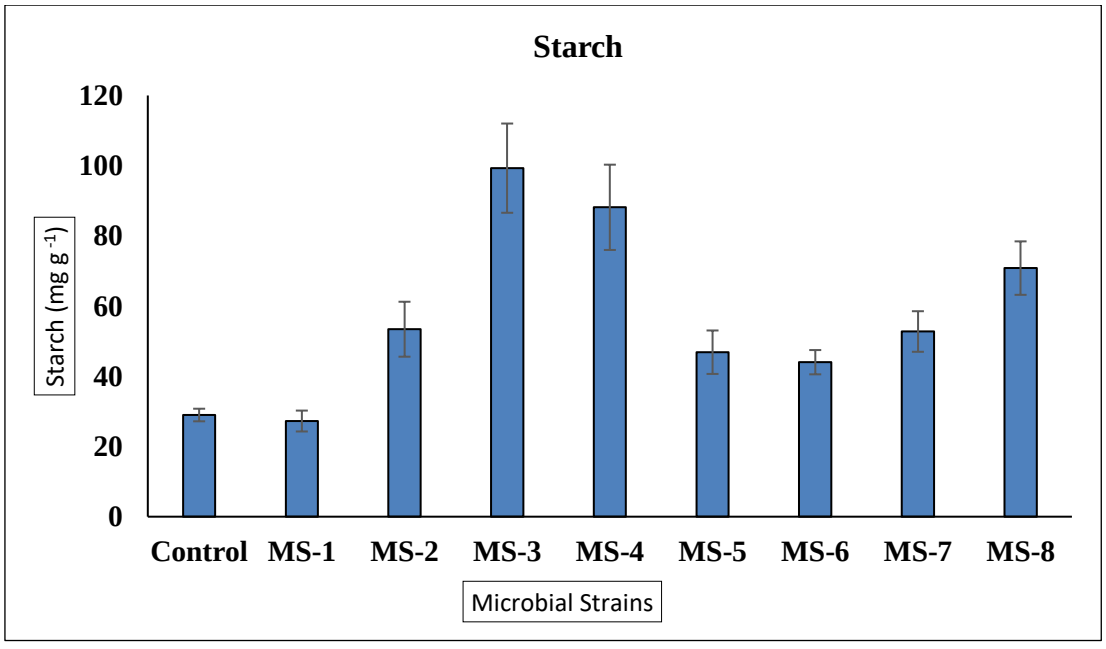

Fig. 3. Concentration of starch in PGPR treated groundnut plant samples.

4.3.2. Soluble sugars analysis:

The increment in the content of soluble sugars was observed in all strains as compared to control except *Agrobacterium sp.* (MS-1) as shown in figure 4. Soluble sugar concentration ranges from 7.6 mg g^{-1} to 27.7 mg g^{-1}. The concentration of the MS-1 strain is 7.6 mg g^{-1} which is lower than the control i.e., 7.9 mg g^{-1}. The maximum concentration of starch is observed in *Ochrobactrum sp.* (MS-3) i.e., 27.7 mg g^{-1}.

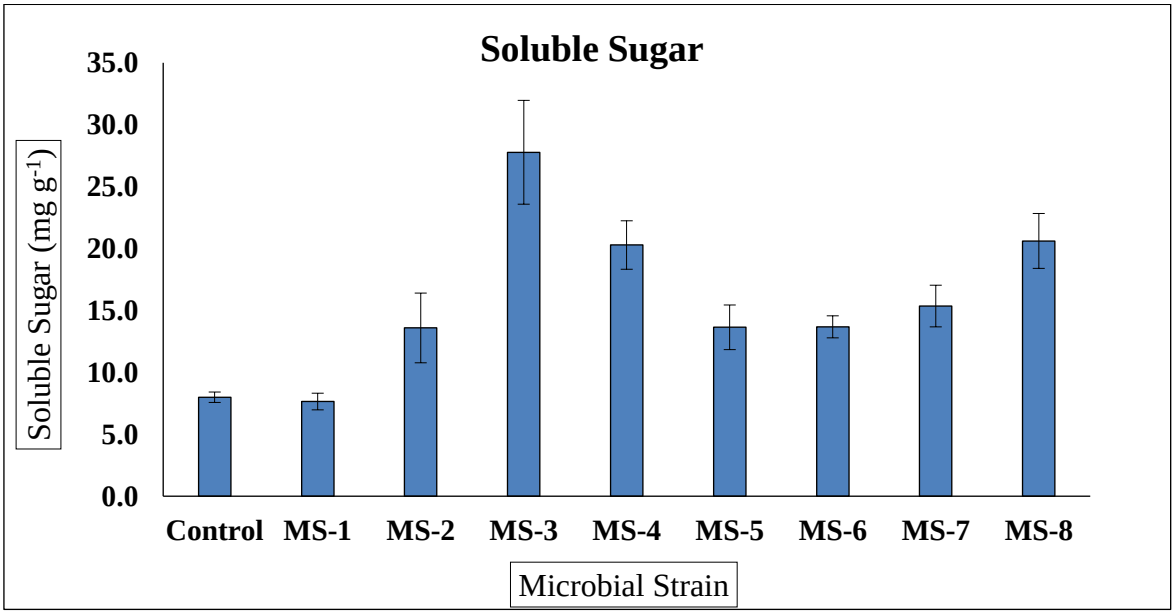

Fig. 4. Concentration of soluble sugar in PGPR treated groundnut plant samples.

4.3.3. Reducing sugars analysis:

The increase in the reducing sugars content was observed in all microbial strains compared to control as shown in figure 5. The content of reducing sugars ranges from 0.84 mg g^{-1} to 1.26 mg g^{-1}. From the given graph it is observed that *Klebsiella sp. III* (MS-7) shows the maximum value i.e., 1.26 mg g^{-1} as compared or control as well as to the other microbial strains.

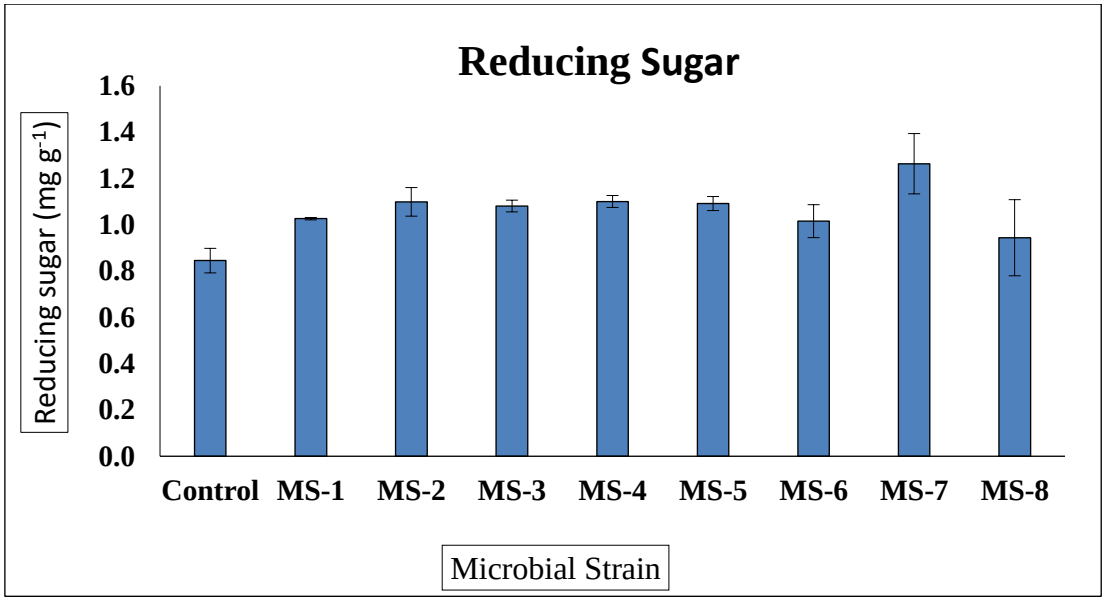

Fig. 5. Concentration of reducing sugar in PGPR treated groundnut plant samples.

4.3.4. Polyphenol analysis:

The concentration of polyphenols in PGPR-treated plants was higher than in control except in *Azospirillum sp.* (MS-8) i.e., 0.67 mg g^{-1}. The range of polyphenol concentration was observed from 0.67 mg g^{-1} to 1.31 mg g^{-1}. The maximum polyphenol concentration was observed in *Klebsiella sp. II* (1.31 mg g^{-1}) as compared to other strains (Figure 6).

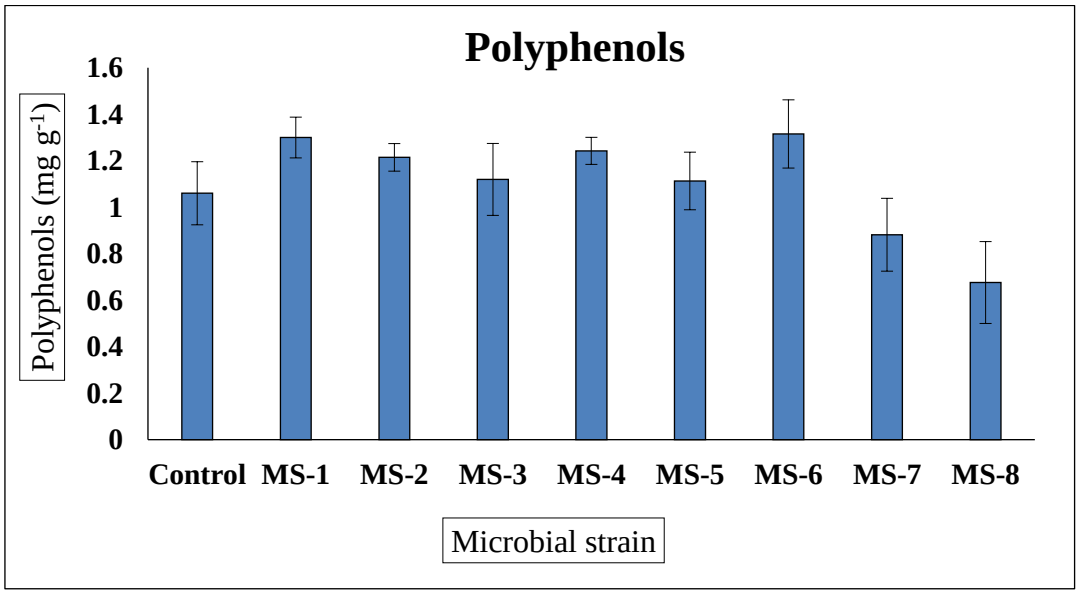

Fig. 6. Concentration of Polyphenols in PGPR treated groundnut plant samples.

Osmolytes are essential in osmotic adjustment during hyperosmotic stress (salinity stress)**.** *Pseudomonas*, *Agrobacterium*, *Klebsiella*, and *Ochrobactrum* are salinity tolerant bacterial strains taken from the roots of halophytes indicating salinity tolerance of 4 to 8% and increased groundnut yield in both saline and control conditions (Egamberdieva et al., 2019). As per the results, *Ochrobactrum* (MS-3) (99.2 mg g^{-1}) had the highest starch content when compared to the other strains. Previous studies demonstrated that groundnut growth and enhancement in salt conditions are improved by halotolerant *Ochrobactrum*. *Pseudomonas* is the most commonly reported genus of PGPR, and isolates from this genus have been proven to provide salt tolerance in a variety of crop species (Sharma et al., 2016).

Total soluble sugars are an important biochemical indicator of salt tolerance in plants. During salt stress, soluble sugars influence 50% of the overall osmotic potential in plant cells (Shukla et al., 2012). Sugar accumulation during salinity is important for osmoprotection, carbon storage, osmotic regulation, and free radical scavenging (Naik et al., 2019). According to the results, all PGPR-treated plants have a significant increase in soluble sugar levels, indicating that the plants' growth and salt tolerance have improved. The accumulation of sugars plays an important function in osmoprotection. Prior studies have shown that *Agrobacterium sp*. has a lower concentration of total soluble than control (Shukla et al., 2012).

Reducing sugars serve a significant function in osmoprotection in groundnut seeds under salinity. Sugars are involved in metabolic activities such as energy production, cell membrane stabilization, turgor maintenance, and signaling, along with osmoprotection (Parida & Jha, 2013). According to the results, reducing sugar concentration increases in all the PGPR strains, this increment helps plants in osmoprotection and osmatic adjustment during high salinity conditions.

Polyphenols are non-enzymatic ROS scavengers in plants that guard plant cells against oxidative damage by enhancing the permeability of the cell membrane (Parida & Jha, 2013). As observed from the results PGPR treated plants under salinity show an increment in the concentration of polyphenols. In response to environmental stresses like salinity, the synthesis, and accumulation of polyphenol increases.

4.4. Antioxidant Enzyme Activity:

4.4.1. Catalase analysis:

The catalase enzyme activity observed in these PGPR-treated groundnut plant samples ranges from 33.1 µ mg^{-1} to 70.2 µ mg^{-1} as shown in figure 7. All of the microbial strains display a decrease in catalase enzyme activity as compared to the control (70.2 µ mg^{-1}). The least concentration was observed in *Pseudomonas sp. II* (MS-5) and *Klebsiella sp. II* (MS-6) i.e., 33.1 µ mg^{-1} and 33.2 µ mg^{-1} respectively.

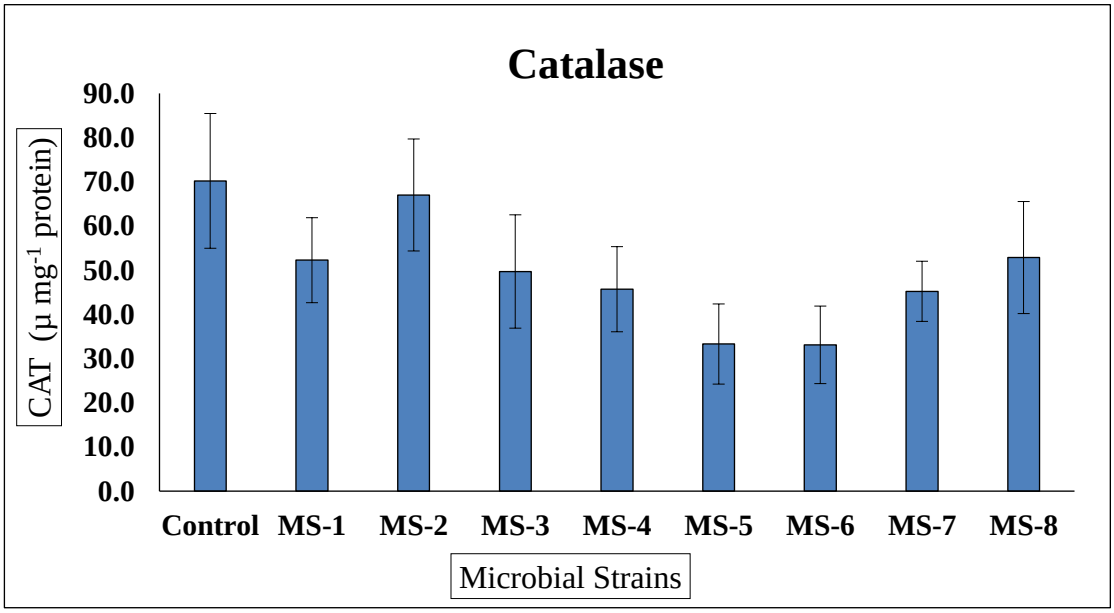

Fig. 7. Effect of catalase (CAT) in PGPR treated groundnut plant samples.

4.4.2. Superoxide Dismutase (SOD):

The concentration of superoxide dismutase (SOD) observed in these plants ranges from 0.19 µ mg^{-1} to 2.64 µ mg^{-1}. There is a significant decrease in the concentration of SOD as presented in figure 8 except in *Pseudomonas sp. I* (MS-4) and *Azospirillum sp.* (MS-8). They showed highest concentration of SOD compared to the control i.e., 2.64 µ mg^{-1} and 2.64 µ mg^{-1} respectively. The least concentration compared to control was observed in *Agrobacterium sp.* (MS-1) which is 0.19 µ mg^{-1}.

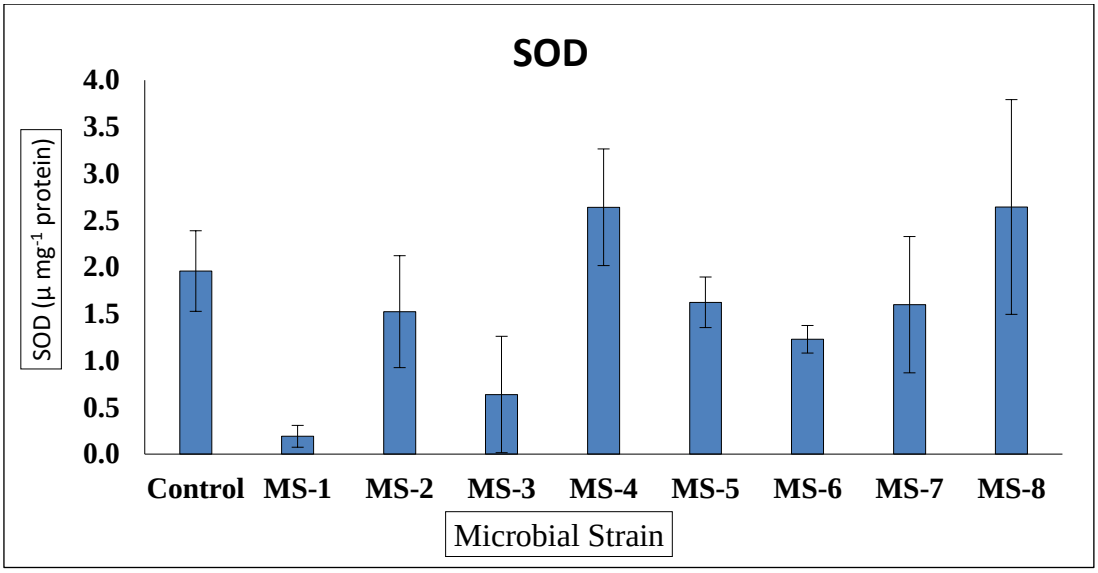

Fig. 8. Effect of SOD in PGPR treated groundnut plant samples

4.4.3. Guaiacol Peroxidase:

As observed in figure 9, the concentration of GPox increases in only one microbial strain i.e., MS-7 (*Klebsiella sp. III*) (4.15 µ mg^{-1}) when compared with control (3.55 µ mg^{-1}) remaining all PGPR treated plants shows a decrease in the concentration of guaiacol peroxidase. The concentration of GPox in these plants samples ranges from 1.66 µ mg^{-1} to 4.15 µ mg^{-1}.

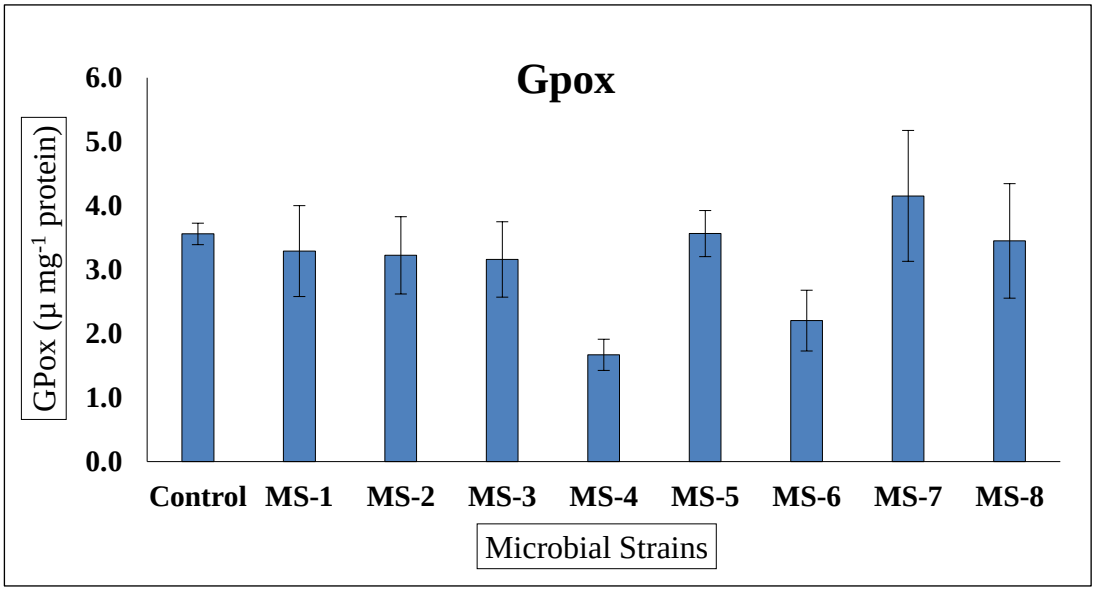

Fig. 9. Effect of GPox in PGPR treated groundnut plant samples

4.4.4. Glutathione Reductase:

The concentration of GR observed in these PGPR-treated plants ranges from 0.15 µ mg^{-1} to 5.77 µ mg^{-1} showing a significant decrease in all strains compared to control as demonstrated in figure 10. The least concentration was shown by *Pseudomonas sp. I* (MS-4) as compared to other bacterial strains.

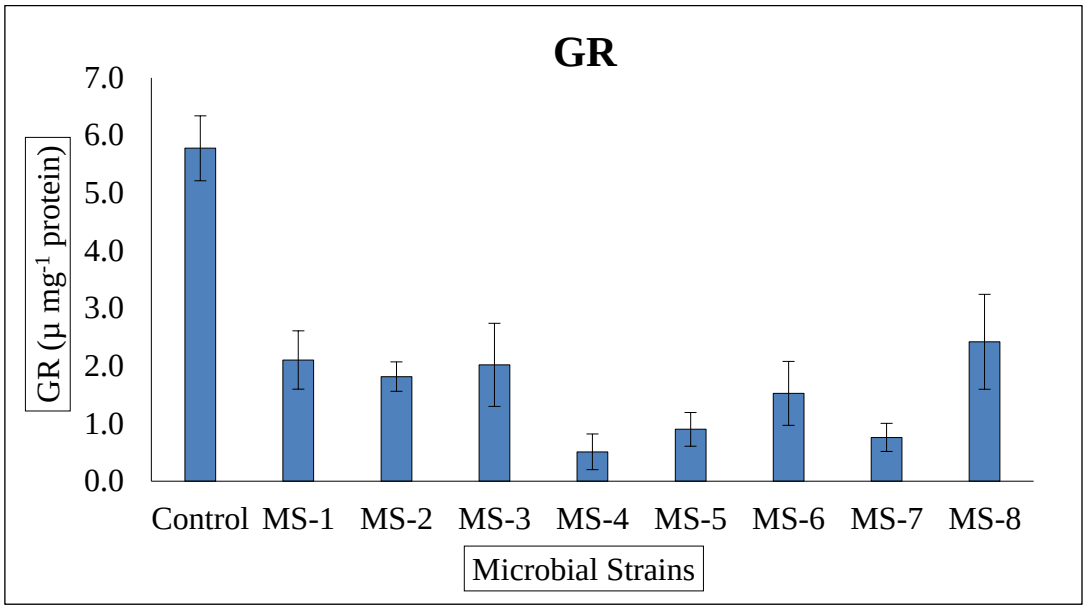

Fig. 10. Effect of GR in PGPR treated groundnut plant samples

When groundnut seeds are exposed to extreme salt conditions, the balance between the ROS production and the scavenging activity of antioxidants may be disrupted resulting in increased membrane lipid peroxidation (Parida & Jha, 2013)**.** Catalase is an essential antioxidant enzyme that has a low affinity for H_2O_2 and is photoinactivated before being damaged when exposed to light. The decline in catalase activity indicates that they are ineffective H_2O_2 scavengers. Catalase activity is also reduced due to severe oxidative stress (Nandal et al., 2015.; Shim et al., 2003). CAT is a ubiquitous enzyme that plays a vital role in cellular defence mechanisms against H_2O_2. It decomposes H_2O_2 into H_2O and O_2 and are present in peroxisomes, cytosol, and mitochondria. In previous studies, it was also observed that in some species of groundnut there was an increase in catalase activity (Chakraborty et al., 2016).

The enzyme SOD is thought to be the first line of defence against ROS, as it aids in the dismutation of O^{2-} to H_2O_2 and O_2, promoting ROS scavenging. The decrease in SOD

indicates a lower capacity for scavenging and dismuting oxygen (Sarker & Oba, 2018). In comparison to control plants, SOD increases scavenging reactive oxygen species. Plants inoculated with *Azospirillum* had higher levels of antioxidant gene expression, which improves antioxidant enzymes and non-enzymatic metabolites, nutrient uptake, growth, and development (El-Esawi et al., 2019). Previous research has shown that an increase in *Pseudomonas* ACC deaminase activity leads to an increase in SOD concentration, which improves salt-resistance and growth (Saravanakumar & Samiyappan, 2007).

Guaiacol peroxidase has been associated with high temperatures, salinity, and drought. It prevents cells from harmful levels of hydroperoxide (Parida & Jha, 2009). Previous research has found that the quantity of GPox in *Klebsiella sp.* increases in wheat plants. The CAT and POX activities can convert the overproduced H_2O_2 caused by salinity into H_2O (R. P. Singh & Jha, 2017). This supports the result, except for *Klebsiella sp.*, which all show a reduction in GPox concentration when treated with salt. By maintaining appropriate H_2O_2 concentrations, this enzyme plays an essential role in the defence mechanisms against oxidative stress.

The decrease in GPox concentration could be related to less H_2O_2 generation. The enzyme glutathione reductase (GR) is required to maintain glutathione's redox stability. The GR has a crucial role in controlling endogenous H_2O_2 content is critical.

(Chakraborty et al., 2016) found a 25% increase in the concentration of GR in groundnut but our result shows a decrease in the concentration of GR in all PGPR treated plants due to less production of H_2O_2.

4.5. Plant Nutrient Analysis:

As shown in Table 4, the carbon content of plants treated with *Klebsiella sp. I* and both the strains of *Pseudomonas* was higher than the control while the remaining strains shows a decrease in carbon content as compared to the control. For nitrogen concentration as compared to the control some PGPR treated groundnut plants (*Agrobacterium sp*., *Klebsiella sp. I, Pseudomonas sp. I,* and *Azospirillum sp*.) show an increment whereas the remaining strains show a decrease in the nitrogen content. Sulphur content shows a decrease in 2 bacterial strains used i.e., *Pseudomonas sp. II* and *Azospirillum.* The maximum concentration of sulphur has been observed in Pseudomonas sp. I (0.61%).

The concentration of Na^+ ions when compared to control increased in 2 PGPR treated groundnut plants (*Agrobacterium sp*. and *Klebsiella sp. I*) whereas slight decrease in the

remaining plants. The concentration of K^+ ions increased in some of the PGPR treated plants (*Pseudomonas sp. I, Klebsiella sp. III,* and *Azospirillum sp.*) as compared to control.

From the nutrient analysis, it was observed that *Pseudomonas sp. I* show maximum C, N, and K^+ content.

The increase in Na (Sodium) concentration indicates a tolerance for possible salinity in the soil solution or substrate, reducing the osmotic or ionic effect on phenological variables like plant height, and weight (Cervantes-Vázquez et al., 2021). One of the most important mechanisms used by PGPR is maintaining a low Na^+/K^+ ratio to promote plant growth under high salinity. Salt stress has been shown to reduce K^+ uptake by lowering intracellular K^+ concentrations.

Table 4: Nutrient analysis of PGPR treated groundnut plants

	C (%)	**N (%)**	**S (%)**	**Na^+ (%)**	**K^+ (%)**
Control	24.45	0.44	0.40	1.33	0.93
MS-1	23.38	0.46	0.40	1.67	0.78
MS-2	24.86	0.45	0.46	1.56	0.74
MS-3	25.33	0.36	0.53	0.87	0.80
MS-4	25.96	0.57	0.57	1.15	1.19
MS-5	21.40	0.40	0.18	1.31	0.89
MS-6	23.49	0.37	0.61	1.31	0.85
MS-7	24.07	0.35	0.46	1.01	1.01
MS-8	20.25	0.50	0.38	1.27	1.00

Conclusion:

From the present study, it has been found that plant growth-promoting rhizobacteria (PGPR) have a high capacity to tolerate various environmental abiotic stresses. It has been observed that PGPR strains treated groundnut plants showed a positive result towards morpho-physiological parameters as compared to control treated groundnut plants. *Azospirillum sp.* shows maximum growth against salinity. These salt-tolerant PGPR withstand high salt concentration by the deposition of compatible solutes such as starch and sugars. It has been observed that *Ochrobactrum sp*., and all strains of *Klebsiella sp.* showed a positive result towards concentration of all compatible solutes as compared to the other strains. Salt stress can also affect the antioxidant enzyme activity of groundnut plants. A high amount of starch and soluble sugars were observed in *Ochrobactrum sp.* whereas the maximum concentration of reducing sugars and polyphenols was observed in the strains of *Klebsiella sp.* By maintaining an appropriate level of H_2O_2 the plants maintain their antioxidant concentrations. There was an increment in enzymatic antioxidant activity of PGPR-treated plants. Among these PGPR strains *Klebsiella sp. I* showed catalase activity, *Pseudomonas sp. I* and *Azospirillum sp.* showed an increment in the activity of Superoxide Dismutase enzyme and *Klebsiella sp. III* shows an increase in Guaiacol Peroxidase activity. The rhizosphere maintains soil fertility by the increase in the nutrient content of the plants. PGPR increases the physiological and nutrient parameters of the soil and influence root colonization. From the nutrient analysis of the given PGPR treated plants, it was observed that plants treated with *Pseudomonas sp. I* show maximum C, Nand K^+ content. Salt tolerant PGPRs of the present study have an adverse mitigated effect against salinity on groundnut seedlings and also improved the seedling growth and germination. In conclusion, the current study opens up the possibility of evaluating the ability of studied halo-bacteria species to alleviate salinity stress in germinating seeds and plant growth under field conditions as biofertilizers. Future research is required for the large-scale production and applications of PGPR.

References:

Abbas, Rameesha, et al. "Halotolerant PGPR: A hope for cultivation of saline soils." *Journal of King Saud University-Science* 31.4 (2019): 1195-1201.

Acosta-Motos, Jose Ramón, et al. "Plant responses to salt stress: adaptive mechanisms." *Agronomy* 7.1 (2017): 18.

Aebi, Hugo. "Catalase in vitro." *Methods in enzymology*. Vol. 105. Academic press, (1984): 121-126.

Ahemad, Munees, and Mulugeta Kibret. "Mechanisms and Applications of Plant Growth Promoting Rhizobacteria: Current Perspective." *Journal of King Saud University - Science* 26.1 (2014): 1–20.

Akhtar, Javaid, et al. "Effect of salinity on yield, growth and oil contents of four Brassica species." *Pakistan Journal of Agricultural Sciences* 39 (2002): 76-79.

Alami, Younes, et al. "Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing Rhizobium sp. strain isolated from sunflower roots." *Applied and environmental microbiology* 66.8 (2000): 3393-3398.

Alexander, Ankita, et al. "Halotolerant PGPR Stenotrophomonas Maltophilia BJ01 Induces Salt Tolerance by Modulating Physiology and Biochemical Activities of Arachis Hypogaea." *Frontiers in Microbiology* 11 (2020): 2530.

Ali, Shimaila, et al. "Amelioration of High Salinity Stress Damage by Plant Growth-Promoting Bacterial Endophytes That Contain ACC Deaminase." *Plant Physiology and Biochemistry* 80 (2014): 160–167.

Amellal, N. et al. "Colonization of Wheat Roots by an Exopolysaccharide-Producing Pantoea Agglomerans Strain and Its Effect on Rhizosphere Soil Aggregation." *Applied and Environmental Microbiology* 64.10 (1998): 3740–3747.

Ansari, Rizwan Ali et al. "PGPR: Current Vogue in Sustainable Crop Production." *Probiotics and Plant Health*. Singapore: Springer Singapore, (2017): 455–472.

Arora, Naveen K. et al. "PGPR for Protection of Plant Health under Saline Conditions." *Bacteria in Agrobiology: Stress Management* 9783642234651 (2012): 239–258.

Ashraf, M., and P. J.C. Harris. "Potential Biochemical Indicators of Salinity Tolerance in Plants." *Plant Science* 166.1 (2004): 3–16.

Backer, Rachel et al. "Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture." *Frontiers in Plant Science* 871 (2018): 1473.

Bano, Asghari, and Mussarat Fatima. “Salt Tolerance in *Zea Mays (L)*. Following Inoculation with Rhizobium and Pseudomonas.” *Biology and Fertility of Soils* 45.4 (2009): 405–413.

Barnawal, Deepti et al. “ACC Deaminase-Containing Arthrobacter Protophormiae Induces NaCl Stress Tolerance through Reduced ACC Oxidase Activity and Ethylene Production Resulting in Improved Nodulation and Mycorrhization in *Pisum sativum*.” *Journal of Plant Physiology* 171.11 (2014): 884–894.

Bhattacharyya, P. N., and D. K. Jha. “Plant Growth-Promoting Rhizobacteria (PGPR): Emergence in Agriculture.” *World Journal of Microbiology and Biotechnology* 28.4 (2012): 1327–1350.

Bradford, Marion M. “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding.” *Analytical Biochemistry* 72.1–2 (1976): 248–254.

Cervantes-Vázquez, et al. “Morphophysiological, Enzymatic, and Elemental Activity in Greenhouse Tomato Saladette Seedlings from the Effect of Plant Growth-Promoting Rhizobacteria.” *Agronomy 2021, Vol. 11, Page 1008* 11.5 (2021): 1008.

Chakraborty, Koushik et al. “Differential Fine-Regulation of Enzyme Driven ROS Detoxification Network Imparts Salt Tolerance in Contrasting Peanut Genotypes.” *Environmental and Experimental Botany* 128 (2016): 79–90.

Chance, Britton, and A. C. Maehly. "[136] Assay of catalases and peroxidases." (1955): 764-775

Chandler, Stephen F., and John H. Dodds. “The Effect of Phosphate, Nitrogen and Sucrose on the Production of Phenolics and Solasodine in Callus Cultures of Solanum Laciniatum.” *Plant Cell Reports* 2.4 (1983): 205–208.

Cohen, Ana C. et al. “Azospirillum Brasilense Ameliorates the Response of Arabidopsis Thaliana to Drought Mainly via Enhancement of ABA Levels.” *Physiologia Plantarum* 153.1 (2015): 79–90.

Connell, James P., and John E. Mullet. "Pea chloroplast glutathione reductase: purification and characterization." *Plant Physiology* 82.2 (1986): 351-356.

Daliakopoulos, I. N. et al. “The Threat of Soil Salinity: A European Scale Review.” *Science of the Total Environment* (2016): 727–739.

Damodaran, T., et al. "Rhizosphere and endophytic bacteria for induction of salt tolerance in gladiolus grown in sodic soils." *Journal of plant interactions* 9.1 (2014): 577-584.

de Lima-Neto, Antonio J. et al. “Papaya Seedlings Irrigation with Saline Water in Soil with Bovine Biofertilizer.” *Chilean Journal of Agricultural Research* 76.2 (2016): 236–242.

Desale, Prithviraj et al. “Plant Growth Promoting Properties of *Halobacillus sp.* and *Halomonas sp.* in Presence of Salinity and Heavy Metals.” *Journal of Basic Microbiology* 54.8 (2014): 781–791.

Egamberdieva, Dilfuza et al. “Salt-Tolerant Plant Growth Promoting Rhizobacteria for Enhancing Crop Productivity of Saline Soils.” *Frontiers in Microbiology* 10 (2019): 2791.

El-Esawi, Mohamed A. et al. “*Azospirillum lipoferum FK1* Confers Improved Salt Tolerance in Chickpea (*Cicer arietinum L.*) by Modulating Osmolytes, Antioxidant Machinery and Stress-Related Genes Expression.” *Environmental and Experimental Botany* 159 (2019): 55–65.

Flowers, Timothy J., et al. “Sodium Chloride Toxicity and the Cellular Basis of Salt Tolerance in Halophytes.” *Annals of Botany* (2015): 419–431.

Gill, Kulvinder. S. “Effect of Soil Salinity on Grain Filling and Grain Development in Barley.” *Biologia plantarum* 21.4 (1979): 241–244.

Glick, Bernard. R. “The Enhancement of Plant Growth by Free-Living Bacteria.” *Canadian Journal of Microbiology* 41.2 (1995): 109–117.

Gondek, Matthew, et al. "Soluble salts in compost and their effects on soil and plants: A review." *Compost Science & Utilization* 28.2 (2020): 59-75.

Gull, Audil, et al. "Biotic and abiotic stresses in plants." *Abiotic and biotic stress in plants* (2019): 1-19.

Gupta, Alka, and Murali Gopal. "Siderophore production by plant growth promoting rhizobacteria." *Indian Journal of Agricultural Research* 42.2 (2008): 153-156.

Gupta, Bhaskar, and Bingru Huang. "Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization." *International journal of genomics* (2014).

Gupta, Govind, et al. "Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture." *J Microb Biochem Technol* 7.2 (2015): 096-102.

Haida, Zainol, and Mansor Hakiman. “A Comprehensive Review on the Determination of Enzymatic Assay and Nonenzymatic Antioxidant Activities.” *Food Science & Nutrition* 7.5 (2019): 1555–1563.

Hamdia, M. Abd El-Samad, et al. “Mechanisms of Salt Tolerance and Interactive Effects of *Azospirillum brasilense* Inoculation on Maize Cultivars Grown under Salt Stress Conditions.” *Plant Growth Regulation* 44.2 (2004): 165–174.

Hansen, Jürgen, and Ib Møller. “Percolation of Starch and Soluble Carbohydrates from Plant Tissue for Quantitative Determination with Anthrone.” *Analytical Biochemistry* 68.1 (1975): 87–94.

Ha-tran, Dung Minh et al. “Roles of Plant Growth-promoting Rhizobacteria (PGPR) in Stimulating Salinity Stress Defense in Plants: A Review.” *International Journal of Molecular Sciences* (2021): 1–38.

Hingole, Shubhangi S., and Anupama P. Pathak. “Saline Soil Microbiome: A Rich Source of Halotolerant PGPR.” *Journal of Crop Science and Biotechnology* 19.3 (2016): 231–239.

Kachhap, Sweta, et al. "Response of plant growth promoting rhizobacteria (pgpr) in relation to elevated temperature conditions in groundnut *(Arachis hypogaea L.*)." *The Ecoscan* 9.3and4 (2015): 771-778.

Kang, Sang-Mo, et al. "Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*." *Journal of Plant Interactions* 9.1 (2014): 673-682.

Kaushal, Manoj, and Suhas P. Wani. "Rhizobacterial-Plant Interactions: Strategies Ensuring Plant Growth Promotion under Drought and Salinity Stress." *Agriculture, Ecosystems and Environment* 231 (2016): 68–78.

Kim, Jongsun, and Douglas C. Rees. "Nitrogenase and Biological Nitrogen Fixation." *Biochemistry* 33.2 (1994): 389–397.

Labuschagne, Nico, T. Pretorius, and Ahamed H. Idris. "Plant growth promoting rhizobacteria as biocontrol agents against soil-borne plant diseases." *Plant growth and health promoting bacteria*. Springer, Berlin, Heidelberg, (2010): 211-230.

Lewis, Edward L., and R. G. Perkin. "Salinity: Its definition and calculation." *Journal of Geophysical Research: Oceans* 83.C1 (1978): 466-478.

Li, H. Q., and X. W. Jiang. "Inoculation with Plant Growth-Promoting Bacteria (PGPB) Improves Salt Tolerance of Maize Seedling." *Russian Journal of Plant Physiology* 64.2 (2017): 235–241.

Lugtenberg, Ben, and Faina Kamilova. "Plant-Growth-Promoting Rhizobacteria." *Annual Review of Microbiology* 63 (2009): 541–556.

Maheshwari, Dinesh Kumar, et al. "Phytohormone-producing PGPR for sustainable agriculture." *Bacterial metabolites in sustainable agroecosystem*. Springer, Cham, (2015): 159-182.

Mayak, Shimon, et al. "Plant Growth-Promoting Bacteria Confer Resistance in Tomato Plants to Salt Stress." *Plant Physiology and Biochemistry* 42.6 (2004): 565–572.

Mehmood, Umair, et al. "A brief review on plant growth promoting Rhizobacteria (PGPR): a key role in plant growth promotion." *Plant protection* 2.2 (2018): 77-82.

Mensah, J. K., et al. "Effects of salinity on germination, growth and yield of five groundnut genotypes." *African Journal of Biotechnology* 5.20 (2006).

Mhatre, Priyank Hanuman et al. "Plant Growth Promoting Rhizobacteria (PGPR): A Potential Alternative Tool for Nematodes Bio-Control." *Biocatalysis and Agricultural Biotechnology* 17 (2019): 119–128.

Miller, Gail Lorenz "Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar." *Analytical Chemistry* 31.3 (1959): 426–428.

Miller, Gad et al. “Reactive Oxygen Species Homeostasis and Signalling during Drought and Salinity Stresses.” *Plant, Cell & Environment* 33.4 (2010): 453–467.

Miller, Gad, et al. “Reactive Oxygen Signaling and Abiotic Stress.” *Physiologia Plantarum* (2008): 481–489.

Misra, Hara P., and Irwin Fridovich. "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase." *Journal of Biological chemistry* 247.10 (1972): 3170-3175.

Mittler, Ron et al. “Reactive Oxygen Gene Network of Plants.” *Trends in Plant Science* 9.10 (2004): 490–498.

Mohan, Deepa, and Anisha Shashidharan. “An In Vitro System for Studying the Effect of Salt Stress in Groundnut (*Arachis Hypogaea L.*).” *Current Biotechnology* 7.6 (2019): 464–471.

Muchate, Niramaya S. et al. “Plant Salt Stress: Adaptive Responses, Tolerance Mechanism and Bioengineering for Salt Tolerance.” *Botanical Review* 82.4 (2016): 371–406.

Munns, Rana et al. “Tissue Tolerance: An Essential but Elusive Trait for Salt-Tolerant Crops.” *Functional Plant Biology* 43.12 (2016): 1103–1113.

Munns, Rana, and Mark Tester. “Mechanisms of Salinity Tolerance.” *Annual Review of Plant Biology* 59 (2008): 651–681.

Naik, Banavath Jayanna et al. “Responses to Biotic and Abiotic Stresses and Transgenic Approaches in the Coffee Plant.” *Journal of the Korean Society of International Agricultue* 31.4 (2019): 359–377.

Naz, Rabia, and Asghari Bano. “MOLECULAR AND PHYSIOLOGICAL RESPONSES OF SUNFLOWER (*Helianthus annuus L.*) TO PGPR AND SA UNDER SALT STRESS.” *Pak. J. Bot* 47.1 (2015): 35–42.

Nehl, D. B., et al. “Deleterious Rhizosphere Bacteria: An Integrating Perspective.” *Applied Soil Ecology* 5.1 (1997): 1–20.

Nelson, Louise M. “Plant Growth Promoting Rhizobacteria (PGPR): Prospects for New Inoculants.” *Crop Management* 3.1 (2004): 1–7.

Pal, Amit Kumar, et al. “Exploitation of IAA Producing PGPR on Mustard (*Brassica nigra L.*) Seedling Growth under Cadmium Stress Condition in Comparison with Exogenous IAA Application.” *Plant Science Today* 6.1 (2019): 22–30.

Palaniyandi, Sasikumar Arunachalam. et al. “*Streptomyces sp.* Strain PGPA39 Alleviates Salt Stress and Promotes Growth of ‘Micro Tom’ Tomato Plants.” *Journal of applied microbiology* 117.3 (2014): 766–773.

Panwar, Meenu, et al. “Native Halo-Tolerant Plant Growth Promoting Rhizobacteria Enterococcus and *Pantoea sp.* Improve Seed Yield of Mungbean (*Vigna radiata L.*) under Soil Salinity by

Reducing Sodium Uptake and Stress Injury." *Physiology and Molecular Biology of Plants* 22.4 (2016): 445–459.

Parewa, Prasad, et al. "Plant growth promoting rhizobacteria enhance growth and nutrient uptake of crops." *Agric Sustain Dev* 2.2 (2014): 101-116

Parida, Asish Kumar, and Bhavanath Jha. "Inductive responses of some organic metabolites for osmotic homeostasis in peanut (*Arachis hypogaea L.*) seedlings during salt stress." *Acta Physiologiae Plantarum* 35.9 (2013): 2821-2832.

Parida Asish Kumar, et al. "Antioxidative Defense Potential to Salinity in the Euhalophyte Salicornia Brachiata." *Journal of Plant Growth Regulation 2009 29:2* 29.2 (2009): 137–148.

Patel, Haridas R., et al. "Impact of projected climate change on groundnut in Gujarat." *Journal of Agro meteorology* 15. special issue-I (2013): 41-44.

Patel, Ravi R. et al. "Alleviation of Salt Stress in Germination of Vigna Radiata L. by Two Halotolerant Bacilli Sp. Isolated from Saline Habitats of Gujarat." *Plant Growth Regulation* 76.1 (2015): 51–60.

Pathak, Rajiv, et al. "PGPR in biocontrol: mechanisms and roles in disease suppression." *Int J Agron Agric* 11.1 (2017): 69-80.

Paulucci, Natalia S. et al. "*Arachis hypogaea* PGPR Isolated from Argentine Soil Modifies Its Lipids Components in Response to Temperature and Salinity." *Microbiological Research* 173 (2015): 1–9.

Podile, Appa Rao, et al. "Plant Growth-Promoting Rhizobacteria." *Plant-Associated Bacteria* (2006): 195–230.

Poonguzhali, Selvaraj, et al. "Cultivation-Dependent Characterization of Rhizobacterial Communities from Field Grown Chinese Cabbage *Brassica campestris sp* Pekinensis and Screening of Traits for Potential Plant Growth Promotion." *Plant and Soil* 286.1–2 (2006): 167–180.

Prasad, Kumar, et al. "Role of PGPR in Soil Fertility and Plant Health." (2015): 247–260.

Prasad, Mahendra et al. "Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable Agriculture." *PGPR Amelioration in Sustainable Agriculture*. Elsevier, (2019): 129–157.

Puniran-Hartley, Norhawa et al. "Salinity-Induced Accumulation of Organic Osmolytes in Barley and Wheat Leaves Correlates with Increased Oxidative Stress Tolerance: In Planta Evidence for Cross-Tolerance." *Plant Physiology and Biochemistry* 83 (2014): 32–39.

Qin, Sheng et al. "Plant Growth-Promoting Effect and Genomic Analysis of the Beneficial Endophyte *Streptomyces sp.* KLBMP 5084 Isolated from Halophyte *Limonium sinense*." *Plant and Soil* 416.1–2 (2017): 117–132.

Rathore, Aditya P., et al. "Phenological and physio-biochemical variations in *Salicornia brachiata* Roxb. under different soil and water treatments (salinity)." *Aquatic Botany* 174 (2021): 103429.

Rojas-Tapias, Daniel et al. “Effect of Inoculation with Plant Growth-Promoting Bacteria (PGPB) on Amelioration of Saline Stress in Maize (*Zea Mays*).” *Applied Soil Ecology* 61 (2012): 264–272.

Roy, Stuart J., et al. “Salt Resistant Crop Plants.” *Current Opinion in Biotechnology* 26 (2014): 115–124.

Ranganayakulu, G. S., et al. "Effect of salt stress on osmolyte accumulation in two groundnut cultivars (*Arachis hypogaea L.*) with contrasting salt tolerance." *African Journal of plant science* 7.12 (2013): 586-592.

Sáenz-Mata, Jorge, et al. "Plant growth promoting rhizobacteria associated to halophytes: potential applications in agriculture." *Sabkha Ecosystems*. Springer, Cham, (2016): 411-425.

Saharan, B. S., and V. Nehra. "Plant growth promoting rhizobacteria: a critical review." *Life Sci Med Res* 21.1 (2011): 30.

Sairam, R. K., and Aruna Tyagi. "Physiology and molecular biology of salinity stress tolerance in plants." *Current science* (2004): 407-421.

Sapre, Swapnil, et al. “*Klebsiella sp*. Confers Enhanced Tolerance to Salinity and Plant Growth Promotion in Oat Seedlings (*Avena Sativa*).” *Microbiological Research* 206 (2018): 25–32.

Saravanakumar, D., and R. Samiyappan. “ACC Deaminase from *Pseudomonas fluorescens* Mediated Saline Resistance in Groundnut (*Arachis Hypogea*) Plants.” *Journal of Applied Microbiology* 102.5 (2007): 1283–1292.

Sarker, Umakanta, and Shinya Oba. “Catalase, Superoxide Dismutase and Ascorbate-Glutathione Cycle Enzymes Confer Drought Tolerance of Amaranthus Tricolor.” *Scientific Reports 2018 8:1* 8.1 (2018): 1–12.

Shaharoona, Baby et al. “Fertilizer-Dependent Efficiency of *Pseudomonas* for Improving Growth, Yield, and Nutrient Use Efficiency of Wheat (*Triticum Aestivum* L.).” *Applied Microbiology and Biotechnology* 79.1 (2008): 147–155.

Sharma, Sandeep, et al. “Halotolerant Rhizobacteria Promote Growth and Enhance Salinity Tolerance in Peanut.” *Frontiers in Microbiology* (2016): 1600.

Shim, Ie Sung et al. “Inhibition of Catalase Activity by Oxidative Stress and Its Relationship to Salicylic Acid Accumulation in Plants.” *Plant Growth Regulation* 39.3 (2003): 285–292.

Shukla, Pushp Sheel, et al. “Improved Salinity Tolerance of *Arachis Hypogaea* (L.) by the Interaction of Halotolerant Plant-Growth-Promoting Rhizobacteria.” *Journal of Plant Growth Regulation* 31.2 (2012): 195–206.

Singh, Amrit Lal, et al. “Screening and Selection of Groundnut Genotypes for Tolerance of Soil Salinity.” *Australian Journal of Crop Science Southern Cross Journals*© 1.3 (2008): 69–77.

Singh, Rajnish Prakash, and Prabhat Nath Jha. "The multifarious PGPR Serratia marcescens CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum L.*)." *PLos one* 11.6 (2016): e0155026.

Singh, Rajnish Prakash, and Prabhat Nath Jha. “Analysis of Fatty Acid Composition of PGPR *Klebsiella sp.* SBP-8 and Its Role in Ameliorating Salt Stress in Wheat.” *Symbiosis* 73.3 (2017): 213–222.

Singh, Rajnish Prakash, et al. “The Plant-Growth-Promoting Bacterium *Klebsiella sp.* SBP-8 Confers Induced Systemic Tolerance in Wheat (*Triticum aestivum*) under Salt Stress.” *Journal of Plant Physiology* 184 (2015): 57–67.

Suzuki, Nobuhiro et al. “Abiotic and Biotic Stress Combinations.” *New Phytologist* 203.1 (2014): 32–43.

Timmusk, Salme et al. “Drought-Tolerance of Wheat Improved by Rhizosphere Bacteria from Harsh Environments: Enhanced Biomass Production and Reduced Emissions of Stress Volatiles.” *PLoS ONE* 9.5 (2014): 96086.

Tiwari, Shweta et al. “Salt-Tolerant Rhizobacteria-Mediated Induced Tolerance in Wheat (*Triticum aestivum*) and Chemical Diversity in Rhizosphere Enhance Plant Growth.” *Biology and Fertility of Soils* 47.8 (2011): 907–916.

Ullah, Asad, et al. "Climate change and salinity effects on crops and chemical communication between plants and plant growth-promoting microorganisms under stress." *Frontiers in Sustainable Food Systems* (2021): 161.

Upadhyay, Sudhir Kumar et al. “Impact of PGPR Inoculation on Growth and Antioxidant Status of Wheat under Saline Conditions.” *Plant Biology* 14.4 (2012): 605–611.

Vardharajula, Sandhya et al. “Drought-Tolerant Plant Growth Promoting Bacillus Spp.: Effect on Growth, Osmolytes, and Antioxidant Status of Maize under Drought Stress.” *Journal of Plant Interactions* 6.1 (2010): 1–14.

Vejan, Pravin et al. “Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability—A Review.” *Molecules* 21.5 (2016): 573.

Vessey, J. Kevin. "Plant growth promoting rhizobacteria as biofertilizers." *Plant and soil* 255.2 (2003): 571-586.

Walia, Abhishek, et al. "Endophytic bacteria: role in phosphate solubilization." *Endophytes: crop productivity and protection* (2017): 61-93.

Wang, Y., and N. Nii. “Changes in Chlorophyll, Ribulose Bisphosphate Carboxylase-Oxygenase, Glycine Betaine Content, Photosynthesis and Transpiration in Amaranthus Tricolor Leaves

during Salt Stress." *Journal of Horticultural Science and Biotechnology* 75.6 (2000): 623–627.

Xu, Yang et al. "Influence of Salt Stress on the Rhizosphere Soil Bacterial Community Structure and Growth Performance of Groundnut (*Arachis hypogaea L.*)." *International Microbiology* 23.3 (2020): 453–465.

Yol, Engin et al. "Characterization of Groundnut (*Arachis hypogaea L.*) Collection Using Quantitative and Qualitative Traits in the Mediterranean Basin." *Journal of Integrative Agriculture* 17.1 (2018): 63–75.

Yousuf, Basit et al. "Application of Targeted Metagenomics to Explore Abundance and Diversity of CO2-Fixing Bacterial Community Using CbbL Gene from the Rhizosphere of *Arachis hypogaea." Gene* 506.1 (2012): 18–24.

Appendix:

ZMB (Zobell Marine Broth)

Ingredients	**g L^{-1}**
Peptic Digest of Animal Tissue	5.0
Yeast Extract	1.0
Ferric Citrate	0.10
Sodium Chloride	19.45
Magnesium Chloride	8.80
Sodium Sulphate	3.24
Calcium Chloride	1.80
Potassium Chloride	0.55
Sodium Bicarbonate	0.16
Potassium Bromide	0.08
Strontium Chloride	0.034
Boric Acid	0.022
Sodium Silicate	0.004
Sodium Fluorate	0.0024
Ammonium Nitrate	0.0016
Disodium Phosphate	0.008
Final pH (at 25°C)	7.6 ± 0.2

TSA (Tryptone Soya Agar)

Ingredients	**g L^{-1}**
Tryptone	15.00
Soya peptone	5.00
Sodium chloride	5.00
Agar	15.00
Final pH (at 25°C)	7.3 ± 0.2

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