Role of bio-inoculants and plant growth substances on soil health, physiology and yield of wheat (*Triticum aestivum* L.) in saline sodic field

TAMOOR UL HASSAN
Ph.D. Plant Physiology

Department of Plant Sciences,
Quaid-i-Azam University, Islamabad
Pakistan
2016
1. **Salinity**

Soil salinization is significantly reducing the area used for agriculture every. Since the earth is facing drastic meteorological changes the magnitude and impact of salinity is increasing. Plants growing under saline conditions suffer dehydration stresses and become vulnerable to diseases.

Among the abiotic constraints, soil salinity is one of the serious environmental hazards limiting the world’s agricultural productivity. Almost all parts of the world are victim of salinity but the effects became drastic in the arid and semi-arid regions. For salinization, altitude does not matter and salt effected lands are sited even in humid tropics and in Polar regions of the world. The problem is dire in developed, and under developed countries which are being deserted due to salinization at very high pace.

It is estimated that about 15% of the total land area of the world is subjected to soil degredation including soil erosion, physical and chemical degradation and soil salinization. The total global area of salt-affected soils including saline and sodic soils is 831 million hectares. 954 million hectares of saline soils is present on the earth's surface. From which 397 million ha are saline and 434 million ha are sodic. The problem is prevalent in all the the continents including Africa, Asia, Australia, and the Americas.

All continents of the world are targeted by salinity. About 80 million hectares of saline soils are in Africa, 357 million hectares in Australasia, 50 million hectares in Europe, 147 million hectares in Central, North and South America. It is believed that about 320 million hectares of land in South and South East Asia is under the grasp of salinity.

In Pakistan, canal system is highly developed and this canal irrigated system exceeded to 62,400 km confined to Indus plain covering an area of 19.43 million hectares (48 million acres). The irrigation water from these canals constantly seeped to the adjacent soils and excessive irrigation
arise the water table between 17-70 cm every year, resulting water-logging and salinity problems. The estimated salt-affected area in the country under cultivation is about 20.9 million hectares plus 10.3 million ha of non-cultivable waste.

The total geographical area of Pakistan is 80.0 million hectares, of which salt affected soils constitute 6.30 million hectares. From which 1.89 hectare is saline, 1.02 million hectare is impermeable saline-sodic, 1.85 million hectare is permeable saline-sodic, and 0.028 million hectare is sodic in nature. Provisionally, 0.45 million hectares are present in Punjab, 0.94 million hectares in Sindh and 0.5 million hectares in NWFP. In Pakistan, out of 19.6 mha of 16 mha land is available for irrigated agriculture and farming.

1.1.2. Types of salt-affected soils

Based on Electrical conductivity, pH and exchangeable sodium ions, salt effected soils are categorized into three types: sodic (or alkali), saline and saline-sodic soils. In saline soil major cations are Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and K\(^+\) and the major anions are Cl\(^-\), SO\(_4\)\(^{2-}\), HCO\(_3\)\(^-\), CO\(_3\)\(^{2-}\) and NO\(_3\). In hyper saline soil B, Sr, Mo, Ba and Al are also present.

a) Saline- sodic soils

Electrical conductivity (EC) of saline sodic soil is greater than 4 dS m\(^{-1}\) and exchangeable sodium percentage (ESP) is more than 15%. The pH value of such soil is less than 8.5. The higher salt concentration which is peculiarity of saline soil and greater exchangeable sodium percentage (ESP) which is salient feature of sodic soil are blended in saline sodic soil.

b) Sodic soils

Sodic soils are dominated by excess sodium on exchange complex along with high concentration of carbonates / bicarbonates. Such soils have high pH (8.5 to 10.7). Sodium absorption ratio (SAR) is always higher which leads to poor soil structure. Biologically, sodic soils are considered dead because water penetration is very low exchangeable sodium ions are very frequent. In Pakistan area affected by sodicity is 7.2 million hectares.
1.1.3. Causes of salinity

Rain fall, weathering of rocks, Wind transported salt (Aeolian) and Poor irrigation of water are the major contributing sources of salinity. Soil solum (the upper part of soil profile) is a part where soluble salts accumulate.

a) Primary salinity

The main causes of this type of salinity are weathering of rocks or oceanic salts. NaCl is the major salt.

b) Secondary salinity

Secondary salinity is due to human activity i.e imbalanced irrigation or change in rain fall pattern and clearing of land. Water table rise gradually in these circumstances and high evapotranspiration to the surface, subsequently result in the formation of salt scald.

1.1.4. Impact of Salinity

Salinity results in low agricultural yield, economy losses, soil erosion, ecological imbalance and accumulation of harmful toxic elements like Se, B.

Different morphological, physiological and biochemical processes of plants are disturbed due to salinity. Salt stress inhibits cell division and reduces shoot growth, decrease dry matter, diminish leaf size and increase root: shoot ratio. Palisade cells become enlarged and leaves become succulent. Photosynthesis is reduced and mineral uptake is affected.

The Ca$^{2+}$ in the medium become inadequate under saline condition. The osmotic signals and ionic Na$^+$ are cues by which plants sense the salt stress. Ligand-gated Ca$^{2+}$ channel on plasma membrane, vacuole and endoplasmic reticulum regulates cytosolic Ca$^{2+}$ during salt stress. The Ca$^{2+}$ serve as second messenger which regulates Na$^+$ transport, and K$^+$/Na$^+$ discrimination in plants.

Salt stress accumulates toxic ions like Na$^+$ and Cl$^-$ which decreases the acquisition of K$^+$ and Na$^+$. Na$^+$ disrupted the binding of Ca$^{2+}$ with cellular components (pectin, phospholipids) and sometime
cause dissociation of Ca$^{2+}$. This dissociation severely affects the integrity of cell membrane and cell wall. Na$^+$ and K$^+$ occupies the same binding sites and being the basic source of activation of cellular enzymes, K$^+$ becomes limited under stress conditions. Salt stress also inhibits high affinity K transporters of K uptake permease (KUP) family and NO$_3$ transporters. The salt tolerance mechanism of plants depends upon some specific physiological and biochemical changes. Among these changes, ion homeostasis to maintain osmotic potential, ion distribution to whole plant and tissue on the basis of water budget are important. The crop varieties generally show higher Na$^+$ and Cl$^-$ transport to shoot and preferential accumulation of Na$^+$ in older leaves under higher salt stress conditions. The uptake of K$^+$, P and Zn become lower with subsequent increase or adjustment of esterase isozyme, compatible solutes and polyamine levels.

Osmotic imbalance and ionic toxicity due to Na$^+$ and Cl$^-$ ions are sensed by plants under salt stress. Some trans-membrane proteins and sensitive enzymes sense excessive Na$^+$ in plant environment. Na$^+$ brings about the conformational changes in membrane depolarization and protein structure.

Plants are stressed in two ways. Firstly water deficit under salt stress caused decrease in water potential due to which cell turgor pressure decreases resulting in plant cell growth inhibition. Secondly salt may enter in transpiration stream and injure cells in transpiring leave resulting in stunted growth.

Under salt stress root growth is less affected than shoot while reduction in dry weight of both root and shoot has been reported. Similarly, harvest index also decreased under salinity.

Halophytes have capability of completing their life cycle in medium containing NaCl because of well adapted morphological, anatomical, and physiological peculiarities. These halophytes follow different mechanisms for abiotic stress including succulence, osmotic adjustment through osmolytes accumulation, ion compartmentalization, maintenance of redox and bioenergetic of
cells, salt inclusion, excretion and selective transport and uptake of ions, enzymatic and non-enzymatic antioxidant response.

In wheat, the seedling emergence and the productive phases (tillering and grain filling) are more sensitive to the specific ions than the anthesis. Under severe salinity stress, not only roots fail to take up water from the soils, but also loose water to the soil. Germination of seeds is often adversely affected by excessive accumulation of salt in the soil.

1.1.6. Mechanism of salt tolerance in plants

Under salt stress conditions, the crop plants either try to avoid the stress, which is indeed not an actual tolerance mechanism or employ the following mechanisms to overcome the salt-induced damages in a sequential adaptations;

1) Plant level transport of salt and its compartmentation
2) Minimize the initial entry of salt from roots
3) Intra cellular compartmentation

a) Initial entry of Salts from Roots

The toxic ions enter into the plant along with the water stream which moves from soil to the vascular system of the root by different pathways like symplastic and apoplastic. Na\(^+\) and K\(^+\) transport is mediated by different transporter which has been clearly demonstrated.

Ion Homeostasis Pathway

Ion homeostasis in cell is taken care of by the ions pumps like antiporters, symporters and carrier proteins on membranes (plasma membrane or tonoplast membrane). Salt Overly Sensitive (SOS) regulatory pathway is one good example of ion homeostasis. This pathway is activated after the receptor perceives the salt stress to alter protein activity and gene transcription by signalling intermediate compounds.

b) Osmoregulation mechanism in plants and microbes
Under salt stress, availability of water become limited and plants readjust their osmotic potential either by reducing water loss or by improving uptake of inorganic solutes. Salt stress inhibits leaf expansion by decreasing turgor which leads to decrease cell wall extensibility.

Most of the plants and bacteria accumulate certain organic solutes such as sugar, alcohol proline, quaternary ammonium compounds in response to osmotic stress which are called osmoprotectants and also termed as compatible solutes because even in high concentration they do not interfere with enzymatic activities. These are localized in cytoplasm and the inorganic ions such as Na$^+$ and Cl$^-$ are preferentially sequestered into vacuole, thus leads to the turgor maintenance for the cell under osmotic stress.

Among higher plants, amino acids like alanine, arginine, glycine, leucine and valine are most important accumulators. The most important osmolyte under salt stress is proline which is imino acid in nature and performs a pivotal role in osmotic adjustment, membrane integrity as well as storage of amino acids. Sugar acts as an osmotica and performs its role in protecting membrane as well as stabilizing membrane structure under salt stress.

Salinity induces accumulation of reactive oxygen species (ROS) which are detoxified by antioxidants activity Salinity brings about higher concentration of ROS such as superoxide, H$_2$O$_2$ and hydroxy-radicals due to the impaired electron transport processes in chloroplast, mitochondria and photorespiration pathways. The level of ROS production reaches to 3-30 folds higher under salinity stress. The reactive hydroxyl radicals can damage vital macromolecules by protein denaturation, mutation and peroxidation of lipids. Plants have devised different systems for scavenging of ROS by using the enzymes like super oxide dismutase (SOD), peroxidases (POD), catalases and antioxidants like ascorbate and reduced glutathione. The plant hormone ABA, is an important signal for the physiological and molecular responses to water-limiting stresses, such as desiccation, salt stress, and cold.
1.1.7. Reclamation of salt-affected soils

In Pakistan, Punjab is hub of agriculture activities and irrigation technology, crop husbandry, mixed cropping, amending the geophysical properties of the sub-soil and soil, leaching with higher level of irrigation water and acid treatment of soil are main causes of salinity. Different methods to combat salinity include gypsum application and surface salt scrapping. Some other measures used combat salinity have also been taken by government including the upgrading of irrigation channels, management of irrigation flows and cultivation of salt tolerant plants.

1.2.1. Physical measures

The porous soils have better prospects for reclamation as they allow maximum infiltration of water with drainage, otherwise, subsequent rise of the water table may result in water logging. In Pakistan most of the salt affected areas have higher percentage of CaCO$_3$ (10-15%). In these soils, exchangeable sodium is transformed into sodium carbonate (Na$_2$CO$_3$) having pH of the salt solution 10.5).

   a) Tillage

Tillage is effective for saline sodic and sodic soils where salt accumulates on the surface leading to puddling and crust formation. This mechanical operation carried out at the time of seed bed preparation. The process improves permeability and water infiltration property of soil.

   b) Deep ploughing

Ploughing from 40-150 cm in stratified soils having impermeable layers improves the physical condition of soil layers and water holding capacity. However deep ploughing is not much effective for saline sodic soils.

b) Addition of sand

For improving root penetration and water permeability, sand is mixed with soil which minimized surface infiltration and facilitates leaching. The combination of deep ploughing
and addition of sand could be better option. The method is labour extensive and not sustainable.

1.2.2. Chemical measures

a) Chemical amendments

Replacement of exchangeable sodium by calcium following addition of chemicals is used to decrease exchangeable sodium percentage (ESP). Gypsum, Calcium chloride, lime + organic manure, sulphur and sulphur containing compounds (sulphuric acid, iron sulphate, aluminium sulphate) are reliable chemicals for neutralizing soil reactions. These chemicals are capable of decreasing sodium absorption ratio (SAR) of saline sodic soil when added with irrigation water. Chemical treatment improved aggregate stability and reduced surface crust formation. However, there are some limitations. The process is expensive and time consuming as well as labour intensive.

a) Mineral fertilizers

The level of salinity can be decreased by excessive fertilizers application, particularly fertilizer containing Ca. The application of mineral fertilizers at suitable time was found to be effective and useful.

However, Chemical fertilizers not only nourish plants and microbes, but also have harmful effects on the soil and its life. The water soluble and concentrated forms of chemical fertilizers are, detrimental and hazardous for soil living organisms. Acidification as well as neutralization of the soil may harm the microbes by depleting soil enzymes which are active at specific pH. Changes in pH slow down enzymes reaction, and microbes undergo dormant or become less active. These microbe form cysts and cannot proliferate due to limitation of nutrients. Chemical
fertilizers containing ammonium sulphate and Superphosphate are strong biocide, because they inhibit nitrogen fixation and kill nematodes and earthworms.

b) Organic, green manures and mulching

Incorporating organic matter improve improve soil permeability and help to release carbon dioxide under salinity. This is accompanied with lowering of soil pH, releasing calcium from CaCO$_3$ through solubilization, thereby facilitating replacement of exchangeable Na with Ca or Mg. The process is helpful in lowering exchangeable sodium percentage (ESP). Mulching reduces evaporation losses thereby decreasing soil salinity. In some developed countries drip irrigation method is also in practise but it is limited for few crops because it is expensive.

1.2.3. Biological measures

a) Phytoremediation

Phytoremediation is application of salt (ion) removing species to control salinity and improve agriculture. Phytoremediation is defined as the application of plants to remove pollutants from soil and environment. Among these, halophytic plant species have pivotal role in reclamation of salt-affected soils. The efficiency of phytoremediation has been proved in the amelioration of sodic and calcareous saline-sodic soils. The salt glands present in the members of some families like Convolvulaceae, (Poaceae), Chenopodiaceae, (Plumbaginaceae), Glaux (Primulaceae and Tamaricaceae are capable of accumulating high salt.

For economic utilization of salt affected areas, cultivation of salt-tolerant crops appear to be one of the best alternatives.

Salt tolerant species of plants like Kallar grass, Sesbania aculeata, Eucalyptus spp, Avicennia marina, Artiplex spp and crops like sugar beet, tomatoes, barley, rye are biologically effective for the remediation of saline soils.

b) Use of Plant Growth Promoting Rhizobacteria (PGPR) and biofertilizers
The soil environment or rhizosphere is a hub of microbial activities which colonize the plants roots and impart positive impact on soil and associated plants. The microbes involved in these activities are called plant growth promoting rhizobacteria (PGPR).

Bacterial biodiversity largely depends upon interaction of plant and soil microbial community. Chemotactic responses of microbiota are based on the stimulations provided by the root exudate containing sugar, amino acid and organic acids.

Plant genotype, agricultural practices as well as cultivation practices influenced the bacterial community and microbial biodiversity in the rhizosphere. Plant age and germination stages are important factors in determining the microbial biodiversity. Microbial biodiversity often increase from seedling to maturation and then declined.

For PGPR, genetics, ecological niche and environment are prerequisite for rhizosphere competence. Rhizosphere competence provides basic platform for effective root colonization with opportunities to proliferate and survive.

A number of bacterial species belonging to genera *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia* are associated with the plant rhizosphere and are able to exert a beneficial effect on plant growth. In plant-microbe interactions, important role is played by plants which select and enrich the bacteria by the constituents of their root exudates.

The roles of phytohormones in ameliorating salinity effects have previously been documented. The IAA producing PGPR impacted positively on growth and yield of different plants coping the deleterious effects of salt. The present work reveals the role of IAA as stressed hormone along with ABA.

Among mutualistic relationship of microorganisms, the vesicular arbuscular mycorrhizal (VAM) symbiosis is most prevalent. Most of plants including Bryophytes, Pteridophytes, Gymnosperm and angiosperms are categorized by VAM. VAM improved the acquisition of mineral nutrients
(nitrogen, phosphorus) and induce tolerance to a wide range of stresses including drought, salt, heavy metals and root-borne pathogens. The application of VAM was beneficial for improving the growth responses of many crops like barley (*Hordeum vulgare*), burgundy (*Macroptilium bracteatum*), wheat and maize.

**Bacillus as PGPR**

Among endospore-forming bacteria *Bacillus* is important for their contribution in agriculture. Physiologically the species of this genus are strong candidates for plant growth promotion and their ability to thrive under adverse climatic and edaphic conditions due to endospore formation and multilayered cell wall composition. Production of peptide signals, peptide antibiotic and extra cellular enzymes also made them compatible. Like other PGPR *Bacillus* spp, have also ability to stimulate plant growth by phosphate solubilization and mobilization, siderophore and phytohormones production, inhibition of ethylene and inducing systematic resistance. Diversity of *Bacillus* is globally widespread. The plant growth promoting ability of these strains have been studied by many researchers. *Bacillus megaterium* is most abundant and exists in various types of soils.

There are growing evidence that *Bacillus* spp, including *B. subtilis*, *B. cereus*, *B. amyloliquefaciens*, *B. pumilus*, *B. pasteurii*, *B. mycoides*, *B. sphaericus*, *P. polymyxa*, *P. azotofixans*, and some other newly discovered species (*B. endophyticus*) have strong role as PGPR and influence the growth, development, and yield of crops under controlled and stressed condition. Seed coating with *Bacillus* sp. on lettuce (*Lactuca sativa* (L.)), (*Phaseolus vulgaris* (L.)), and cucumber (*Cucumis sativus* (L.)) enhanced seed storage and half-life.

*Bacillus megaterium* application on wheat enhances N and Phosphorus. The consortium of *Bacillus erythropolis*, *Bacillus pumilus*, *Bacillus* sp, *Bacillus subtilis*, and *Pseudomonas rubiacearum* was effective on *Lactuca sativa* L. (Lettuce) when applied as biofertilizer. *Bacillus pumilus* 8N-4 has the potential as PGPR and reported to improve yield of wheat. observed that
**Bacillus edaphicus** NBT strain was helpful in improving K content of rape and cotton. Biofertilizer comprising **Bacillus mucilaginosus** and **Pseudomonas fluorescense** strains were evaluated on spring barley, winter and spring wheat, potato, and sugar beet in field under different soils in Central Russia.

c) **Pseudomonas as PGPR**

**Pseudomonas** is ubiquitous bacteria in agricultural soils and has many traits that make them well suited as PGPR. The most effective **Pseudomonas** sp is **Fluorescent pseudomonas**. Considerable research is underway globally to exploit the potential of **Fluorescent pseudomonas**. The **Pseudomonas fluorescense** plays an effective role in stimulating yield and growth traits of chickpea and sugarcane as reported previously. Specific strains of the **Pseudomonas fluorescens** and **Pseudomonas putida** have recently been used as seed inoculants on crop plants to promote growth and. The occurrence and activity of **Pseudomonas** is affected by a variety of environmental factors (e.g. soil type, nutrient abundance, pH, moisture content) as well as plant-related factors (species, age).

Application of **pseudomonas** spp improved plant growth, agronomic characteristics of plants and induced yield.

d) **PGPR and salinity stress**

The application of **Pseudomonas chlororaphis** TSAU13 and **Pseudomonas extremorientalis** TSAU20 as bioinoculants enhanced the protein contents and yield of soybean and common bean in Uzbekistan.

An increase in salinity in the soil causes a physiological response or disorder in lettuce plants. The long-term goal of improving plant–microbe interactions for salinity affected fields and crop productivity can be met with an understanding of the mechanism of osmoadaptation in **Azospirillum** sp. The synthesis and activity of nitrogenases in **A. brasilense** is inhibited by salinity stress. There is a close relationship found between the phosphate solubilising activity and
low pH levels in the growth medium which suggests that phosphate solubilization is the result of organic acids released from bacterial metabolism.

*Pseudomonas* spp, rapidly colonize plant roots of *Zea mays*, *Gossypium hirsutum*, *Triticum aestivum*, and potato, and cause statistically significant yield increases in field test under salinity stress.

The salt tolerant PGPR produced exopolysacchride (EPS) to protect them from harmful effects of salinity. Similarly, the accumulation of poly-β-hydroxybutyrate and proline accumulation enhanced the salt tolerance potential of PGPR.

e) **Carrier based bioinoculants i.e biofertilizers**

According to Vessey (2003) “Biofertilizers are substances which contain living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant, and promote growth by increasing the supply or availability of primary nutrients to the host plant”.

Food demands are increasing day by day in Asia and owing to population growth eco-friendly agriculture in the form of biofertilizer is ideal and suitable solution. Biological nitrogen fixation contributes 180 X 10^6 metric tons/year globally, out of which symbiotic associations’ produces 80% and the rest comes from free-living or associative systems. The ability to reduce and derive such appreciable amounts of nitrogen from the atmospheric reservoir and enrich the soil is confined to bacteria and Archaea.

Improvement in soil fertility through fixing atmospheric nitrogen, making the P available through P- solubilization and production of plant growth substances are the main outcome of biofertilizer. Long term use of biofertilizers is efficient, productive, and economical as well as eco-friendly.
The use of bio-fertilizer and bioenhancer such as N-fixing bacteria and beneficial microorganism can reduce chemical fertilizer applications and consequently lower production cost. Utilization of PGPR in order to increase the productivity may be a viable alternative to organic fertilizers which also helps in reducing the pollution and preserving the environment in the spirit of an ecological agriculture. PGPR or combinations of PGPR and AMF can improve the nutrient use efficiency of fertilizers and allow reduced application rates of chemical fertilizers.

The Nitragan” Rhizobia was first biofertilizer formulated in USA in 1985. In 1937 Azotobacter comprising formulation “Azotogen” appeared in Russia. The extensive research on Biofertilizer started since 1975 and one major milestone was achieved by exploring Azospirillum lipoferum inoculum “Azogreen” for maize in France (1994).

The Effects of PGPR based biofertilizer (BioGro) had significant on growth and yield of rice in Vietnam.

In Pakistan Ayub Agriculture Research Institute was pioneer when formulated “Bej ka tika” in 1965. Bio power (1996) was formulated at NIBGE (National institute of Biotechnology and Genetic Engineering Faisalabad), Biozote (1998) at NARC (National Agriculture Research center), rice Biofert at University of Faisalabad, and Humiphos, Biophos (2010) by COMSATS Institute of Information Technology have been formulated.

1.1. Plant growth substances

1.2.1 Tryptophan and its role in plant growth

Tryptophan (C\textsubscript{11}H\textsubscript{12}N\textsubscript{2}O\textsubscript{2}) which is found in L and R- enantiomers, is among one of the 22 essential amino acids of human diet. It is encoded by UGG codon and only L- stereoisomers of tryptophan are active in structure of enzymes and proteins with indole as functional group. On industrial scale tryptophan is produced by indole and serine by using bacteria (B. amyloliquefaciens, B. subtilis, C. glutamicum or E. coli) in the presence of enzyme tryptophan-synthetase.
L-tryptophan application as physiological precursor of indole acetic acid (IAA) and its effects on plant growth and physiology has been demonstrated by many. *Azospirillum* sp, and *Rhizobium, Bacillus amyloliquefaciens* FZB42 and *Fluorescent pseudomonas* application as PGPR with L-tryptophan have played important role for improvement of physiology and yield of different plants.

Tryptophan is abundantly available along with sucrose in the soil and rich amount of tryptophan is available near the root tips (12-16 cm). Dead and decaying parts of plants, leaf litter and root exudates of plants are the main sources of tryptophan.

Application of L-tryptophan with PGPR in soil has proved very fruitful for increasing growth of different plants like under normal and under salt stress. The growth and productivity of wheat has also been effected by the combination of PGPR and L-tryptophan under salt stress.

(L-tryptophan)

**1.2.2. Putrescine**

Putrescine, is an organic compound which is chemically known as tetramethylenediamine (C₄H₁₂N₂). It is produced by break down of amino acids and on industrial scale it is prepared by succinonitrile.

The involvement of putrescine in plant growth, development, cell division, differentiation, and embryogenesis has been reported. Putrescine is also part of the group of aminoacids which are highly responsive to environmental stresses, especially salt stress. They are reported to increase
the nutrient acquisition thereby influenced on of proline, protein and nucleic acid synthesis as well stabilized cell membranes under salt.

Putrescine

The leaf litter and dead decaying plant material are sources of proteins and amino acids like tryptophan which mostly occurs under salt stress. The ABA is stress hormone and induce tolerance to plants. Recent research demonstrates the involvement of indole acetic acid (IAA) in improving salt tolerance of plants. However, the mechanism of salt tolerance induced by tryptophan has not been investigated yet.

The root exudate contained variety of chemical compounds including tryptophan (L-tryptophan) which is converted into IAA by PGPR. L-tryptophan is naturally present in root exudates of plants or synthesized by hydrolysis of proteins of dead cells. The tryptophan is converted to indole acetic acid which is accomplished by the activity of plant growth promoting rhizobacteria

Study areas

The saline sodic field was provided by Soil salinity Research Institute Pindibhatian, district Hafizabad (maximum average temperature = 23°C, rainfall = 7 mm. and relative humidity = 60.11%) while the un-stressed field, pots experiments under stress and unstressed axenic condition was conducted at Quaid-i-Azam University Islamabad (maximum average temperature = 21.9°C, rainfall = 8.98 mm and relative humidity = 67.16%) Islamabad.
Aims and Objectives

The present investigation was based on the hypothesis that PGPR from stressed habitat can impart tolerance to plants when used as bioinoculants. The persistence of PGPR in the rhizosphere soil and the limited shelf life of the biofertilizers are the major constraints for the (farmers in the commercialization of bioferilizers. The comparative role of two carriers maize straw and sugarcane husk) with PGPR in improving the PGPR proliferation, and alleviating salinity stress was checked. The role of putrescine a polyamine in the salt tolerance of plants has been investigated to compare the effects with that of PGPR mediated salt tolerance. In order to achieve these goals following experiments were conducted.

1) The isolation of bacterial strains was made from two of halophytic herbs (*Chrysopogan aucuheri* and *Cincharis cilaris*) due to abundance, availability, and rich C -source of roots. Selection of root powder from halophytes was aimed to evaluate the role of root powder as carrier for the PGPR and further to procure better tolerance to temperature, moisture and salt stresses.

2) PGPR produce phytohormone and modulate the level of phytohormone in plants. The efficiency of these PGPR were checked in the presence of 1) tryptophan a precursor of IAA a plant growth promoting hormone as well as 2) with carriers material in the form of biofertilizer

3) The effects of PGPR isolates *Bacillus cereus* and *Pseudomonas moraviensis* in different combinations with two different carrier materials (maize straw and sugarcane husk) for the formulation of carrier based fertilizer for saline lands.

4) Since *Pseudomonas moraviensis* wild type strain has ability to synthesise IAA in the presence and absence of tryptophan and IAA has pivotal role under salt. The affectivity and ability of *Pseudomonas moraviensis* for conversion of tryptophan to IAA was determined. Tansposon
mutagenesis was used to construct transposon insertion mutant library of IAA deficient mutants. The transposon mutants were screened out on the basis of tryptophan conversion into IAA.

5) The effects of foliar spray of putrescine on the physiology and yield of wheat crop under salt stressed and unstressed, pot and field conditions.

Chapter 2

2. Introduction

Halophytes are capable of completing their life cycle in medium containing NaCl and other salts because of well adapted morphological, anatomical, and physiological attributes. Halophytes follow different mechanisms to cope with salt stress including succulence, osmotic adjustment, ion compartmentalization, maintenance of redox and energetic status and selective transport and uptake of ions, enzymatic and nonenzymatic antioxidant. Adaptation to salt stress in halophytes is mediated by ion homeostasis, osmolyte biosynthesis, scavenging of toxic radicals, water transport long distance transport and co-ordinated response to stimuli.

Halophytes are the major contributors of endophytic bacteria and PGPR. Isolation of 29 endophytic strains from *Prosopis strombulifera*, some of these were positive for phytohormones and siderophore production. *Salicornia brachiata* hosted variety of endophytic microflora in roots. Recently, 20 PGPR were isolated from four different halophytes (*Cressa cretica, Salicornia brachiata, Suadea nudiflora* and *Sphaeranthus indicus*) and most of them were efficient phytohormone producers.

Pakistan has vast salt range descending from Kalabagh to Jhelum. Khewra salt range surrounds Khewra salt mine which is the second largest salt mine in the World. Higher concentration of Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl and HCO\(_3\), SO\(_4\)\(^{2-}\) were reported in the rhizosphere soil of plants growing in Khewra salt range.
Khewra salt range of Pakistan comprises rich floral diversity of the low lying sub-tropical forests. Both legumes and non legumes are common in this area but *Olea ferruginea* (Royle) and *Acacia modesta* (L.) are dominant trees. *Kochia indica* (Weight.), *Suaeda fruticosa* (Forsk.), *Salsola foetida* (Del), *Haloxylon multiflorus* (Bunge.), and *Herniaria hirsute* (L.) Pers are the common perennials while *Sporobolus arabicus* (Boiss.) and *Cynodon dactylon* (L.) Pers are dominant herbs. *Dodonea viscosa*, *Justica adhatoda*, *Lantana indica*, *Lespedeza floribunda* and *Opuntia monocantha* are the dominant shrubs. The herbal vegetation of Khewra comprises *Heteropogon contortus*, *Chrysopogon serrulatus*, *Dicliptera bublenroides Pupalia lappaceae* and *Dicanthrium aunnlatum*.

*Cenchrus ciliaris* (Buffel grass), the member of family Poaceae is perennial herb of Asian and African region. Plant reaches to 50 cm in length at maturity and spike appeared during flowering season. Deep rooting system and higher biomass production, facilitate plant to cope with drought and deserted condition. It has strong antibacterial activity against some bacterial strains and plays dominant role in carbon sequestration, Nitrogen cycling and soil binding. *Cenchrus ciliaris* has potential to survive at high salt concentration because of its strong antioxidant activities and osmolytes production.

*Solanum surattense* (Yellow-Berried Nightshade), the member of family Solanaceae is naturally growing prostrate herb in central Asia is considered as important halophyte of Pakistan. The plant bear purplish flower throughout the year. Chemical constituents of *Solanum surattense* are of great medicinal values. This weed contains high antioxidant activity and phenolic contents even when grown in natural unstressed soil.

*Chrysopogon aucheri* (Aucher's grass) is a member of family Poaceae and widely distributed in Asia and Africa. It is perennial herb (30-60cm in height) blossoms during June and July. *Chrysopogon aucheri* leaves accumulate higher Na and Ca contents. Agriculturally important
Root colonizing halophytic bacteria have also been isolated from *Chrysopogan aucheri*, growing in salt stress range.

*Aerva javanica* (kapok bush), is perennial herb belongs to family Amaranthaceae and commonly found in sandy soils. Plant height varies from 0.4 - 1.8 m) and often blossomed with white flowers between January to October. *Aerva javanica* branches are used as tooth paste.

*Peaganum harmala* (Syrian rue), the member of family Zygophyllaceae is perennial herb and known for its medicinal values. It is often used for its diuretic, analgesic, disinfectant, anthelmintic and anti-inflammatory activities. The endophytic PGPR in the roots were isolated from prairie or crop plants. Endophytic PGPR inoculation proved effective in root development, improving the growth, physiology and yield of many crops.

PGPR are the most economic and beneficial source to increase plant growth, speed up seed germination and increase yield by protecting plants from deleterious environmental stresses. However the persistence and survival efficiency of these PGPR under natural field condition are limited and variable.

The *Burkholderia*, *Enterobacter*, *Azospirillium*, *Azotobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* are commonly used in agriculture. The strains belonging to *Bacillus*, *Pseudomonas* and *Stenotrophomonas* have also been evaluated for agronomic yield improvement.

The survival efficiency and stimulation of biological activities of PGPR under salt stress depends upon the salt tolerance potential of strains, ion selectivity and osmoregulation mechanism. The salt tolerant PGPR produced exopolysaccharide (EPS) to protect them from harmful effects of salinity. Similarly, the accumulation of poly-β-hydroxybutyrate and proline accumulation enhanced the salt tolerance potential of PGPR.
*Bacillus cereus* served as plant growth promoter and is a strong antifungal agent and P-solubiliser hence effective as bio pesticide against the fungal pathogens that attack nodulation stage of Pigeon Pea. *Bacillus cereus* was affective in increasing the yield, growth and nutrition of broccoli plant under organic growing conditions.

The importance of *Pseudomonas moraviensis* as potential source in seed germination and yield improvement has been reported previously. Consortia of *Pseudomonas moraviensis* and other microbial strains improved growth and yield of chickpea. Improvement in root and shoot biomass of sugarcane was observed when treated with *Pseudomonas moraviensis*. The *Pseudomonas* effects on winter wheat were dependent on developmental phase of crop and on population size of inoculum.

*Stenotrophomonas* acts as a bio control agent and is a strong producer of antibiotic (Hayward et al., 2010). Comprehensive role of *Stenotrophomonas* as plant growth promoting rhizobacteria has also been documented previously.

Application of Plant Growth Promoting bacteria (PGPB) isolated from halophytes may be a potential source for crop improvement growing under salt stress. Present attempt was aimed to evaluate the role of root powder along with endophytic bacteria from halophyte herb *Cenchrus ciliaris* on soil fertility status and plant growth under saline sodic condition of the field and compared with the single salt (NaCl) effects in potted plants grown under axenic condition. The second part of this experiment was conducted under unstressed condition both in pots as well as in field, using root powder of *Cenchrus ciliaris*.

The halophyte root powder harbouring the halotolerant bacteria has never been evaluated as mean for the amelioration of salt stress. The purpose was twofold. Firstly, whether the efficiency of PGPR residing in roots is augmented when added with root powder. Secondly, the moisture and temperature tolerance of the PGPR inside the roots as the shade dried root powder was used.
2.1. Material and methods

2.1.1. Plant material and growing condition

Geographically Khewra mines are located at the foot hills of salt range between longitudes 07300, 26.9 E and latitudes 3239, 03.4 N, 288 m.a.s.l. This salt range hosts a large number of halophytes species. The roots and rhizosphere soil of these halophytes harbor a diverse group of microorganism (Khalid, 2010).

During the present study plants were collected from saline soil of Khewra salt range [pH: 8.5; EC: 2.3 dSm\(^{-1}\); 32°56′00″N (North latitude); 73°44′00″E (East longitude); annual rain fall: 900 mm] from an altitude of 300-395 m.a.s.l with minimum human interference area.

The buffle grass (*Cenchrus ciliaris* L.) a naturally grown halophyte was uprooted when the plant was 13-15 cm high at vegetative stage. The roots of the plants was washed with tap water followed by washing with sterilized water, shade dried for 5-7 d at room temperature and ground into powder form. Three microbes were isolated from the root powder.

2.1.2. Field Experiment

Seeds of *Triticum aestivum* (L.) cv Inqlab 91 were obtained from Soil salinity Research Institute Pindibhatian. Prior to sowing seeds were surface sterilized with 70% ethanol for 5 min and then seeds were soaked in 10% chlorox for 2-3 min followed by successive washing with autoclaved distilled water.

Seeds were grown in saline sodic soil (EC= 4.76 dSm\(^{-1}\)) of field measuring 10 m\(^2\) at Soil Salinity Research Institute Pindibhattian (maximum average temperature = 23\(^\circ\)C, rainfall = 7 mm and relative humidity = 60.11%). Seeds were also planted in the unstressed normal field soil (EC= 0.43 dSm\(^{-1}\), pH 8.8 = and silt: clay: sand in 14:11:75) at Quaid-e- Azam University, Islamabad (maximum average temperature = 21.9\(^\circ\)C, rainfall =8.98mm and relative humidity = 67.16%).
Distance between two rows was 36 cm. The RCBD design was used in field. Among all the halophytes *Cenchrus ciliaris* root powder had significantly higher protein, sugar and organic matter. The treatments consisted of *Cenchrus ciliaris* root powder (T) and untreated control. The root powder was sieved through 0.20-0.312 mm IS sieve and added to the saline field by hand drill method at the rate of 100 g/10 m². No chemical or organic fertilizer was added in the soil. Plant sampling was done at early vegetative stage (57 DAS) for physiological parameters and at maturity (159 DAS) for yield parameters.

### 2.1.3. Pot Experiment

Pots experiment was conducted at Quaid-e-Azam University Islamabad. Earthen pots measuring 17x20 cm² were filled with garden soil and mixed with sand in 3:1 ratio. In each pot root powder was mixed with 8 Kg soil (autoclaved twice at 121 °C temperature and 1.03 lb pressure) at the rate of 20 g/pot. No chemical or organic fertilizer was added. In each pot 10-15 seeds were sown and 5 plants/pots were maintained till maturity. Salinity was induced by adding 150 mM NaCl (EC= 3.6 dSm⁻¹) 3d after seed germination.

### 2.1.4. Isolation of endophytic microbes

For isolation of endophytic bacteria, root powder (1 g) was suspended in 9 ml autoclaved distilled water and an aliquot (100 µl) from decimal dilution was spread on LB agar plates. The culture plates were incubated for 24-72 h at 27°C. The number of viable cell counts at 10⁷ dilution was calculated following the formula.

Viable cell count (cfu/g) = (number of colonies/volume of inocula) x dilution factor

### 2.1.5. Viable cell count (cfu/g soil) from root powder

The cfu/g soil and root powder was calculated by the formula:
Viable cell count (cfu/g) = Number of colonies/Volume of inoculum × dilution factor

2.1.6. Sampling and Physico-chemical analyses of rhizosphere soil

The rhizosphere soil samples of wheat were collected at early vegetative stage (57 DAS) below 7-10 cm from surface. Soil samples were ground, and sieved through 2 mm sieve and processed for the isolation of rhizobacteria and determination of physico-chemical properties.

2.1.7. Soil pH, electrical conductivity (EC) and organic matter

Electrical Conductivity (EC), pH and organic matter content of soil were measured prior to sowing and at 57 DAS (2-3 leaf stage).

2.1.8. Nitrate-N (NO$_3$-N) and phosphorus (P)

Soil samples were extracted for Nitrate-N (NO$_3$-N), Phosphorus (P) and bi-carbonate ions following the method. Nitrates (NO$_3$-N) in the fresh leaves were measured by spectrophotometer (Shimadzu UV-1208, Shimadzu Co., Kyoto, Japan) at 410 nm.

2.1.9. Analysis of physiological parameters

The SPAD (Minolta reading SPAD 502, Germany) was used for the measurement of leaf chlorophyll content. Fully expanded young grown leaves were selected for the measurements (Rahimi, et al., 2010).

2.1.10. Protein content of leaves

Protein content of leaves (57 d old plants) was determined by the method. Fresh leaves (0.1 g) were ground in phosphate buffer (pH 7.5), centrifuged for 10 min at 3000 rpm. The supernatant (0.1 ml) was taken in test tube and final volume (1ml) was made by adding distilled water. Sample was treated with Folin Phenol reagent and absorbance of each sample was recorded at 650 nm after 30 min incubation.
2.1.11. Sugar estimation

Sugar contents of leaves and roots powder was measured. Homogenate (0.5 g plant tissue + 10 ml distilled water) was centrifuged at 3000 rpm for 5 min. Supernatant (0.1 ml) was treated with 5 ml concentrated sulphuric acid. After incubation (4 h) absorbance was recorded at 420 nm.

2.1.12. Proline estimation

Proline content of leaves was measured by the method. Plant material (0.5 g) was homogenized in 10 ml of 3% aqueous sulphosalicylic acid. Filtrate (2 ml) was boiled with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h. The reaction mixture was extracted with 4 ml toluene and stirred for 15-20 sec. The absorbance of toluene layer was read at 520 nm against toluene blank.

2.1.13. Extraction for antioxidant enzymes

Fresh leaves (5 g) were homogenized with 15 ml of 0.05 N phosphate buffer (pH 7.0) containing 10% polyvinyl poly pyrrolidone and 0.1 M Ethylene diamine tetra acetate (EDTA).


Extraction of antioxidant enzymes was made following the method. The assay mixture for peroxidase contained 0.1 ml enzyme extract, 1.35 ml of 100 mM MES buffer (pH 5.5), 0.05% \( \text{H}_2\text{O}_2 \) and 0.1% phenylenediamine. Change in absorbance was recorded at 485 nm with spectrophotometer (UV-120-01, Shimadzu). The activity of POD was expressed as \( \Delta \text{OD} 485 \text{nm} \cdot \text{min} \cdot \text{mg}^{-1} \) protein. Superoxide dismutase (SOD) activity was determined by measuring inhibition of photochemical reduction of nitroblue tetrazolium (NBT) using method of Beauchamp and Fridovich, (1971).

2.1.15. Determination of phytohormones

The extraction and purification for ABA and IAA from the leaves were made following the
Plant leaves (1 g) were grounded in 80% methanol at 4°C with an antioxidant, butylated hydroxyl Toluene (BHT). The leaves and roots were extracted at 4°C in dark for 72 h with subsequent change of solvent. The extracted sample was centrifuged and the supernatant was reduced to aqueous phase using rotary thin film evaporator (RFE). The pH of aqueous phase was adjusted to 2.5-3.0 and partitioned four times with ½ volume of ethyl acetate. The ethyl acetate was dried down completely using rotary thin film evaporator. The dried sample was re-dissolved in 1ml of methanol (100%) and analyzed on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) using UV detector and C-18 column (39x300 mm) for identification of hormones. Samples (100 µl) were filtered through 0.45µ millipore filter were injected in column. Pure ABA, IAA and GA3 (sigma, USA) was used as standard for identification and quantification of hormones. ABA, GA and IAA were identified on the basis of retention time and peak area of standards. Methanol, acetic acid and water (30: 1: 70) were used as mobile phase. The flow rate was adjusted at 0.5 ml/min with an average time for 22 min/sample. The detection of IAA was made at 280 nm and ABA was detected at 260 nm. For GA analysis wavelength was also adjusted at 254 nm.

2.1.16. DNA Extraction

Extraction of genomic DNA of bacterial strain was carried out by using the Gen Elute Bacterial Genomic DNA Kit as described by.

**PCR amplification**

The genomic DNA of PGPR was amplified by the method. The polymerase chain reaction (PCR) was carried out by using forward (fd1) primer having nucleotide sequence AGAGTTTGATCTGGCTCAG and reverse (rd1) primer (AAGGAGGTGATCCAGCC). The
reactions were carried out in a thermocycler (Biometra, Germany). Each reaction volume (25µl) contained 1µl of template DNA, 0.2 mM dNTP mix, 1.5mM MgCl₂, 5 µl of 10 × taq buffers, 1 unit of Taq DNA polymerase and 10 pmols of each primer. The volume was raised to 25µl by autoclaved cold water. After denaturation at 95°C for 2 min, 30 rounds of temperature cycling (94 °C for 30 sec, 55 °C for 30 sec and 72 °C for 2 min) were followed by incubation at 72 °C for 10 min. Then, 5µl of amplified PCR products were electrophoresed on 1.2% (w/v) agarose gel, in 1 X TBE buffer at 80 V and then stained with ethidium bromide (0.01g/mL). Gel was visualized under UV transilluminator lamp (S. N. 76S/64069, Bio RAD, Italy) and photographed. 1Kb DNA ladder (Fermentas, Germany) was used as marker. The PCR products was excised from gel and was purified by using gel purification kits (JET quick, Gel Extraction Spin Kit, GENOMED) and sequenced on automated sequencer. The sequences were compared with standard databases by BLAST (NCBI) software.

**Sequencing for 16S rRNA**

The purified PCR products of approximately 1,400 bp were sequenced by using 2 sets of primers 27F AGA GTT TGA TCM TGG CTC AG; 1492R TAC GGY TAC CTT GTT ACG ACT T; 518F CCA GCA GCC GCG GTA ATA CG; 800R TAC CAG GGT ATC TAA TCC. Sequencing was performed by using Big Dye terminator cycle sequencing kit v.3.1 (Applied Bio Systems, USA). Sequencing products were resolved on an Applied Bio systems model 3730XL automated DNA sequencing system (Applied Bio Systems, USA) at the Macrogen, Inc., Seoul, Korea.

2.1.19. Cost benefit ratio analysis

The benefit cost ratio per hectare was calculated by formula explained.

\[ BCR = \frac{\text{Value of gross production} - \text{cost of inputs (investments)}}{\text{cost of inputs (investments)}} \]
The cost of inputs = \( C_{sd} + C_{fert} + C_{Hrp} + C_{pp} + C_{lab} + C_{land} + C_{irrig} + C_{Misc} \)

Where;
\( C_{sd} = \) cost on seed
\( C_{fert} = \) cost on fertilizer
\( C_{Hrp} = \) cost on halophyte roots (labour + grinding + transportation)
\( C_{pp} = \) cost on plant protection
\( C_{lab} = \) cost on labour
\( C_{land} = \) cost on land preparation
\( C_{irrig} = \) cost on irrigation

2.1.15. Statistical design

Statistical analyses of the data were conducted using analysis of variance (ANOVA) in Statistix program, version 8.1. Complete Randomize Design (CRD) and Randomized Complete Block Design (RCBD) were followed for pots and field experiment respectively. Four replicates were taken and mean value (four replicates of each year) were presented in tables. Mean values were separated according to LSD test (p = 0.05) with ±SE.

2.2. Results

2.2.1. Molecular characterization of endophytic PGPR from Cenchrus ciliaris

Among six halophytes used for screening; the root powder of Cenchrus ciliaris was rich in soluble proteins, sugar and organic matter contents (Table 2.1).

The three PGPR, identified from genomic DNA analysis were as follow.

For the isolate (1) obtained from roots of Cenchrus ciliaris the total length of sequence with 1540 nucleotide was obtained. The comparison of the nucleotide sequence with data nucleotide bank showed 99% sequence similarity for 1477/1482 nucleotide bases with that of Bacillus cereus strain MSU AS 16S ribosomal RNA gene, partial sequence (ACC No:LN714048).
For the isolate (2) obtained from roots of *Cenchrus ciliaris* the total length of sequence with 1535 nucleotide was obtained. The comparison of the nucleotide sequence with data nucleotide bank showed 99% sequence similarity for 1477/1485 nucleotide bases with that of *Pseudomonas moraviensis* isolate PSB34 16S ribosomal RNA gene, partial sequence (ACC No:LN714047).

For the isolate (3) obtained from roots of *Cenchrus ciliaris* the total length of sequence with 1540 nucleotide was obtained. The comparison of the nucleotide sequence with data nucleotide bank showed 99% sequence similarity for 1466/1472 nucleotide bases with that of *Stenotrophomonas maltophilia* strain 2681 16S ribosomal RNA gene, partial sequence (ACC No: LN714049).

### 2.2.2. Colony Forming Unit (cfu/g root powder)

The cfu of PGPR in root powder, measured at 65 d old root powder which was stored at room temperature had higher values (24% and 20%) for *Bacillus cereus* and *Pseudomonas moraviensis* respectively over *Stenotrophomonas maltophilia* (Fig 2.1). The cfu of all three PGPR declined at 130 d as compared to cfu recorded at 65 d. The greater decline (50%) was observed in *Pseudomonas moraviensis*. *Bacillus cereus* and *Pseudomonas moraviensis* had 19% and 10% higher cfu at 130 d than *Stenotrophomonas maltophilia*.

### 2.2.3. Survival Efficiency of plant growth promoting bacteria (PGPR) in rhizosphere of wheat

The survival efficiency of PGPR measured as cfu/ g rhizosphere soil of wheat (Fig 2.2), made at early vegetative stage of plant (57 DAS) was higher for *Bacillus cereus* and *Pseudomonas moraviensis* than *Stenotrophomonas maltophilia*. At 122 DAS (booting stage), the cfu was declined significantly by 56% in *Bacillus cereus* and *Pseudomonas moraviensis*, and 25% in *Stenotrophomonas maltophilia*. 
4.2.5. Effects of halophyte root powder on physicochemical properties, macronutrients and micronutrients under salt stressed conditions.

During the first year (2010-2011), no significant effects of halophyte root powder was recorded in pH of rhizosphere soil both in pots and field experiment (Table 2.3 and Table 4.4). Similarly, addition of root powder imparted no significant effect on EC and SAR of the rhizosphere soil of potted plants but there were 15% and 20% decreases in EC and SAR respectively in rhizosphere soil of field grown plants. EC and SAR were 3% and 5% higher over control in field grown plants over potted plants. The organic matter of rhizosphere soil in field grown plants and potted plants was significantly higher (19% and 29% respectively) receiving root powder treatment. Similar trend was observed in EC, pH, SAR and organic matter during 2011-2012.

Under salt stressed condition, increase in K\(^+\) and P was significantly (30-40%) higher both in pots (Table 2.3) and field (Table 2.4) grown plants however NO\(_3\)-N was higher only in pots grown plant. Under salt stressed condition field and potted plants exhibited equal (8%) increase in P content while NO\(_3\)-N content was 20% higher in field grown plants. Potted plants exhibited 11% increase in K content over field grown plants.

Increase in Mg\(^+\) was 30% and 75% in pots and field grown plants respectively. Sodium absorption ratio (SAR) was 20% less in field grown plants over control. Similar trend was observed for NO\(_3\)-N, P, K, Ca and Mg contents in 2011-2012.

During 2010-2011 under salt stressed condition the Na content was significantly decreased in the rhizosphere soil of field grown wheat plants. But no significant effects of root powder were observed in the Cl and HCO\(_3\) contents of rhizosphere soil of either in field or pot grown plants. Increase in Cl was 10% greater in field grown plants over potted plants. Similar trend was followed in 2011-2012.
During 2010-2011, the Na⁺/K⁺ ratio was reduced by 21% and 33% in the rhizosphere soil of field grown (Table 2.3) and potted (Table 2.4) plants. The Na⁺/Ca⁺ ratio was also decreased by 24% in the rhizosphere of field grown plants but no significant effect of root powder was detected in the rhizosphere of potted plants. Similar to the rhizosphere soil, the leaves of the plant also showed decline in the Na⁺/K⁺ and Na⁺/Ca⁺ ratio in response to root powder addition. The magnitude of decrease was significantly higher (83%) in leaves of potted plants as compared to that of field grown plants (19%). Similar trend was observed during 2011-2012.
2.2.4. Effects of halophyte root powder on physicochemical properties, macronutrients and micronutrients under unstressed soil.

Under unstressed condition during 2010-2011, soil organic matter was 10% higher in field grown plants. Organic matter was significantly increased by 85% and 31% respectively in rhizosphere soil of potted (Table 2.5) and field (Table 2.6) grown plants receiving root powder treatment. Increases in organic matter of unstressed rhizosphere soil were 56% and 12% in potted and field grown plants respectively over stressed soil of potted and field grown plants. Similar trend was observed in organic matter during 2011-2012.

Significant increases in NO$_3$-N and P (58% and 35%) were observed in rhizosphere soil of pots containing sterilized soil. The % increase in NO$_3$-N and P were 28% in potted plants, grown under unstressed condition over the potted plants grown under stressed condition. In field grown plants, increase in NO$_3$-N was equal (10%) over control, under stressed and unstressed condition. However, increase in P content was 17% higher in unstressed field grown plants. The P content was increased by 36% and 24% over control in potted and field grown plants.

Similarly, 44% and 20% greater K was recorded in potted and field grown plants respectively. Increases in K content of unstressed rhizosphere soil were 25% and 13% in potted and field grown plants respectively over stressed soil of potted and field grown plants.

Mg contents were significantly higher both in pots (Table 2.5) and field (Table 2.6) grown plants under unstressed condition during 2010-2011. Mg contents of the soil were increased by 35% in field and 75% in pots over control. In field grown plants increase in Mg was 33% over control under stressed and unstressed condition. In potted plants increase in Mg content was 9% higher under unstressed condition. Under stressed condition 5% greater Ca content was recorded in potted plants. Increases in Ca content of unstressed rhizosphere soil were 5% in potted and field
grown plants over stressed soil of potted and field grown plants. Similar trend was observed for organic matter, NO$_3$-N, K, Ca, and Mg contents during 2011-2012.

### 2.2.5. Effects of halophyte root powder on nutrient accumulation of leaves under stressed condition

During 2010-2011, the leaves of potted plants grown under stressed condition and treated with halophyte root powder exhibited 26% higher NO$_3$-N and 58% higher P over control. The increases in NO$_3$-N and P were 33% and 23% in field grown plants respectively (Table 2.7).

During 2010-2011 under salt stress condition leaves K was significantly (35 - 50%) higher in the potted and field grown plants (Table 2.7) however, Percentage increase was higher in potted plants. Under stressed condition field grown plants exhibited 4% increase in Ca content over potted plants. Increase in Ca accumulation was significantly (55%) higher over control in potted
Increase in Mg content was significantly (23%) higher in field grown plants. Under stressed condition field grown plants exhibited 17% increase in Mg content over potted plants. Halophyte root powder decreased accumulation of Na in leaves by 42% in potted plants. % decrease in Na was 32% in potted plants over field grown plants. Similar trend was observed in 2011-2012.
2.2.6. Effects of halophyte root powder on nutrient accumulation of leaves under unstressed condition

Under unstressed condition during 2010-2011, accumulation of NO$_3$-N was 39% and 31% higher in pots and field grown plant (Table 2.8) respectively, while phosphorus contents were 36% and 57% higher in field and pots grown plants grown plants respectively. Increase in K content was 50% and 39% higher in potted and field grown plants. The K contents were 38% and 22% higher in potted and field grown plants under unstressed condition over potted and field grown plants grown under stressed condition.

Halophyte root powder treatment enhanced Ca content of leaves in equal magnitude (50%) in potted and field grown plants. The Ca contents were 12% and 32% higher in potted and field grown plants under unstressed condition over potted and field grown plants grown under stressed condition. The accumulation of Mg was significantly (63%) higher in the leaves of potted plants while this increase was 31% in field grown plants (Table 2.8). The Mg contents were 41% and 8% higher in potted and field grown plants under unstressed condition over potted and field grown plants grown under stressed condition. During 2011-2012 similar trends were observed for NO$_3$-N, P, K, Ca and Mg contents.
2.2.8. Effects of halophyte root powder on growth and physiology of wheat under stressed condition

During the first year (2010-2011), the height of the plants (Table 2.9 and Table 2.10) was 20% and 26% higher over control in potted and field grown plans. Under stressed condition potted plants exhibited 4% increase in plant height over field grown plants. During second year 2011-2012 the pattern of increase in plant height was similar to that of the first year.

Increase in fresh weight of the aerial parts was 23% higher over control in potted (Table 2.9 and Table 2.10)) and field grown plants. Increase in fresh weight was similar (22%) over control in potted and field grown plants under stressed condition.

During 2010-2011 increase in chlorophyll contents were significantly (30%) higher in potted and 36% in field grown plants under salt stress condition (Table 2.9 and Table 2.10). Chlorophyll contents of stressed plants were increased by 9% and 24% in potted and field grown plants respectively over potted and field grown unstressed plants. Under stressed condition field grown plants exhibited 7% increase in Chlorophyll content over potted plants.

Protein contents of plants treated with root powder were also significantly (46%) higher both in field and potted plants as compared to control. Increase in protein content was equal (38%) over control in potted and field grown plants.

During 2010-2011 sugar contents were significantly (30-35%) higher both in potted and field grown plants (Table 2.9 and Table 2.10). Increase in sugar content was equal (32%) over control in potted and field grown plants.

Proline content of halophyte root powder treated plants was increased significantly (45%) over control in potted and 61% in field grown plants. Proline contents of stressed plants were increased by 27% and 22% in potted and field grown plants respectively over potted and field
grown unstressed plants. Under stressed condition field grown plants exhibited 16% increase in proline content over potted plants.

During 2010-2011 in the field grown and potted plants (Table 2.9 and Table 2.10) under salt stressed condition, superoxide dismutase (SOD) activity was 41% and 28% over control. There was no significant difference in % increase of SOD activity in potted and field grown of stressed and unstressed plants. Under stressed condition field grown plants exhibited 13% increase in SOD activity over potted plants.

The peroxidase (POD) activities were significantly (31%) higher over control in potted plants while this increase was 34%. In field grown plants POD activity was 39% higher under stressed condition. Under stressed condition field grown plants exhibited 12% increase in POD activity over potted plants. Similar trend was found in 2nd year trial.

Under salt stress during 2010-2011, all the three hormones IAA, GA and BA were higher in the leaves of plants treated with root powder. Noteworthy, the ABA content in field grown plants (Table 2.10) was much higher than that of potted plants (Table 2.9). There was significant increase of IAA by 60% and 35% in leaves of field and pots grown plants respectively. GA and ABA contents were increased by 38% and 39% in field grown plants while 57% and 35% increases were recorded in pot grown plants over control. Similar trend was obtained in 2010-2011.

4.2.7. Effects of halophyte root powder on growth and physiology of wheat under unstressed condition

During 2010-2011, under unstressed condition significantly higher increase 30% and 27% in plant height was observed over control in potted and field (Table 2.11) grown plants respectively. Increases in plant height were 3% and 5% higher in potted and field grown plants respectively.
over potted and field grown plants under stressed condition. Under unstressed condition, the fresh weight was significantly increased by 27% and 30% during 2010-2011 in potted plant and field grown plants respectively (Table 2.11). Increases in fresh weight were 5% and 10% in potted and field grown plants respectively over potted and field grown stressed plants. Similar trend was observed in 2011-12.

Chlorophyll content was significantly increased by 26% and 20% over control in field grown plants and potted plants, grown under unstressed condition (Table 2.11). Proline content were significantly (18% and 39%) higher in potted and field grown plants respectively (Table 2.11).

During 2010-2011, the superoxide dismutase (SOD) activity of halophyte root powder treated leaves was 26% and 41% higher over control in potted and field grown plants respectively (Table 2.11). The peroxidase (POD) activities were 31% and 58% higher over control in potted and field grown plants (Table 2.11). Similar trend was observed in 2011-2012.
2.2.9 Effects of halophyte root powder application on yield of wheat under salt stressed condition

During 2010-2011 halophyte root powder addition improved spike length, seeds/spike and seed weight by 29%, 23% and 15% respectively in potted plants (Table 2.12). Increases in yield parameters were similar during 2011-2012. Spike length was 18% and 16% higher in potted and field grown plants under stressed condition over potted and field grown plants under unstressed condition. Under stressed condition field grown plants exhibited 17% increase in spike length over potted plants.

Contrary to field grown plants, potted plants produced 24% greater seeds under stressed condition. Under stressed condition field grown plants exhibited 19% increase in seed number over potted plants.

Seed weight was 8% higher in field grown plants over potted plants under salt-stressed condition. Under salt-stressed condition field grown plants exhibited equal magnitude of increase (7%) in seed number in potted and field grown plants.

During 2010-2011, halophyte root powder increased the number of plants/m², spike length and seeds/spike by 46%, 47% and 43% over control under field condition (Table 2.12). Increase in plant/m² was 30% higher in field grown plants under stressed condition over field grown plants under unstressed condition.
2.9 Effects of halophyte root powder application on yield of wheat under unstressed condition

Under unstressed conditions during 2010-2011, halophyte root powder addition improved number of plant/m² by 16% over control in field (Table 2.13). Increases in spike length, seeds/spikes were 11% and 28% in potted plants (Table 2.13). In field grown plants increases in spike length, seeds/spikes were 30% and 18% respectively. Number of seeds were 4% higher in unstressed field grown plants over potted plants. Potted plants treated with halophyte root powder exhibited equal magnitude in seed weight 10% over control, under stressed and unstressed condition.
2.2.10. Benefit cost ratio

The cost benefit ratio for the production of per hectare yield were 1.33 and 1.39 for wheat grown in saline sodic field and unstressed field respectively. These results indicate that halophyte root powder application may increase the farmer’s benefits by 33% and 39% in salt stress and unstressed condition respectively.

2.3. Discussions

The survival efficiency of *Pseudomonas* and *Bacillus* in pure culture with NaCl has previously been reported. The higher cfu obtained in the rhizosphere of wheat inoculated with halophyte root powder harbouring *Pseudomonas moraviensis* and *Bacillus cereus* residing as endophytes in roots may be attributed to the availability of C/N sources present in root powder. The thermal resistance of *Bacillus cereus* spore to germinate and grow under adverse environmental conditions. The organic matter, protein and sugar contents of the root powder may serve as additional C/N sources for the indigenous microorganisms in the field. The plethora of microorganisms acts synergistically to sequester the salts in their cells and hence decrease the EC and SAR of rhizosphere soil in the field.

The improved nutrients status in the rhizospheric soil under sterilized pots and field condition and the selective ions accumulation in leaves following the application of root powder of halophyte were reported. The associated PGPR (*Pseudomonas moraviensis*, *Stenotrophomonas maltophilia* and *Bacillus cereus*) residing there in the root powder possibly assists nutrients availability in soil and further regulates their translocation to the leaves.

The decrease in toxic ions (Na, Cl and HCO₃⁻) accumulation due to the application of halophyte root powder in the field was beneficial and induced the reclamation of saline land.
Reduced accumulation of toxic ion might be a strategy of PGPR to sequester salt or to modulate $\text{Na}^+/\text{H}^+$ pump to combat stress. Under unstressed condition soil structure, nutrient storage, and biological activity depend upon availability of carbon and organic matter contents. The increase in P-solubilization due to PGPR application has been reported to improve growth and yield of many plants. It is well documented that P-solubilization ability of PGPR enhanced uptake of P, K, Ca and Mg positively affecting plant growth.

The magnitude of decrease in EC and SAR, and toxic ions in soil and the translocation of these ions to leaves were more pronounced in the field grown plants treated with root powder. Noteworthy the effect of root powder was less pronounced in potted plants grown under induced salt stress using single salt (NaCl). Possibly the PGPR ($\text{Bacillus cereus}$, $\text{Pseudomonas moraviensis}$, and $\text{Stenotrophomonas maltophilia}$) residing in the root have better tolerance to salt and high temperature. In field plethora of microbes resides which may minimize the effect of salt stress. Since their potential as bioinoculants on wheat did not decrease even after shade drying or grinding to powder. This is evidenced by the maintenance of their activity even after shade drying at room temperature and grinded to powder.

Similarly under unstressed condition, nutrient improvement in rhizospheric soil and their selective accumulation by the plants treated with root powder was recorded. The PGPR residing inside the root powder might assist the nutrients availability and their translocation. Both the Na/K and Na/Ca ratio were decreased in the rhizosphere of field grown plants. This was in contrast to the higher decrease in the Na/K ratio of the rhizosphere soil of potted plants. These observations may be accounted for the differences in the salt composition (NaCl, CaCO$_3$, Na$_2$CO$_3$) and the interactive effects of edaphic and climatic factors in the field. The field grown plants exhibited decrease in the plant fresh weight at early vegetative phase, contrary to the potted plants showed decrease at late vegetative phase. This suggests that field grown plants cope with the dehydrative stress induced by the salinity at early
vegetative phase and was able to modulate turgidity at late vegetative phase. This early
decrease in the fresh weight of aerial parts of field grown plants treated with root powder
could be an adaptive mechanism of the treated plant for salt tolerance.

Increased accumulation of NO\textsubscript{3}-N and Mg in sterilized soil and natural field was recorded.
Nitrogen and Mg are integral part of chlorophyll structure insinuate toward higher
chlorophyll contents of leaves of treated plants. Decline in soluble sugar and protein content
in mature leaves was correlated with lower chlorophyll contents and enhanced leaf
senescence under salt stress.

Soluble sugar and protein play important role in plant growth because they assist in osmotic
adjustment and protect macromolecules from degradation. Observed increase in proline and
sugar contents contributes to the osmotic adjustment and protect macromolecules degradation
under environmental stresses.

Proline contents and antioxidant enzymes activities were higher in field grown plants than
potted plants because in field diverse microbial community influenced the rhizosphere and
assist the plants.

Salinity results in excess accumulation of reactive oxygen species (ROS) because in such
conditions cellular homeostasis is disrupted. Antioxidant enzymatic system is necessary for
detoxification of ROS (reactive oxygen species) but reduction in antioxidant activities were
observed in PGPR treated wheat. Detoxification of these ROS is achieved via non-enzymic
and enzymic antioxidative system. Increase in antioxidant activities due to antioxidant
promoting system induced by PGPR application decreases deleterious effects of reactive
oxygen species. Lower activity of antioxidants at later stages of plants growth is correlated
with respiratory rate and energy metabolism requirement as described earlier. The POD
activity was higher at late vegetative phase which suggest that the scavenging system of H\textsubscript{2}O\textsubscript{2}
produced as a result of ROS detoxification by SOD was more efficient at late vegetative stage.
in potted plants. Both the scavengers (SOD and POD) and detoxification system were more effective in the field grown plants treated with root powder.

PGPR are involved in the modulation of plant growth regulating hormones (auxin, cytokinin and gibberellins) in plants and affect plants growth and development at different. This is indicated by the observed increase in the growth hormones in root powder treated plants.

The detection of higher ABA content in field grown plants may be an adaptive strategy to cope with the salinity stress in the saline sodic soil of the field. The increase in ABA content of treated leaves which is positively correlated with efficiency of PGPR for improving ABA in culture. During the adverse environmental conditions ABA act as signalling molecule for survival of plants. ABA increased xylem water potential and water uptake under salt stress and act as root-to-shoot signal. Exposure of plants to salinity stress resulted in increased level of ABA and it is correlated with leaf and soil water potential. Addition of root powder increased the leaf ABA 1AA, and GA production in plants in present study. The increase level of ABA impart positive role in acclimation of salinity by improving antioxidative mechanism. It is well documented that enhanced level of phytohormones and increased level of nutrients can positively affect plant growth and PGPR are prime source of this increase.

Increase in yield parameter of halophyte pieces treated plants containing PGPR might be attributed to efficient uptake of water, nutrients as well as increased rate of photosynthesis. The increase in yield under salinity stress can also be attributed to higher photosynthetic activities of treated plants followed by efficient uptake of water and nutrients. *Pseudomonas* and *Bacillus* associated with roots (endophytes) accelerate the nutrient availability and acquisition, thereby substantially increased the yield of cereals. Under natural condition, *P. moravensis* strain was found to be a beneficial source as bioinoculant for improving banana growth and production.

**2.4. Conclusions**
In conclusion, the halophytic root powder harbour the PGPR *Pseudomonas moraviensis, Bacillus cereus Stenotrophomonas maltophilia* which can survive at higher room temperature, and can tolerate salinity and mechanical stress of grinding. The added halophyte root powder ameliorated the salinity effects by decreasing EC, SAR and Na in treated soil which was more pronounced in saline sodic field grown plants. Plants grown under unstressed condition (pot or field) had better growth than plants grown under stressed condition. Protein content was markedly increased in field grown plants and under unstressed condition while proline, antioxidants and ABA was increased under stressed condition. The magnitude of increase in yield components was higher in field grown plants than potted plants. The root powder of halophytes can be a better option as carrier for biofertilizer formulation also to enrich the soil with organic matter. The need is to re-inoculate the root powder (used as carrier) with the PGPR residing there, in order to get synergistic action and better performance as biofertilizer.

**Chapter - 3**

3. Introduction

Phytohormones are important for plant production exerting direct effects on physiology and growth of plants. Auxin is considered as most important phytohormone for its prominent and diverse role in plants growth and physiology.

Among several strategies adopted by PGPR for improving growth and physiology of plants, the modulation and alteration of hormonal balance is of prime importance. The phytohormones are effective both in normal and under stressed conditions. Salinity often reduced the production of phytohormones (auxin, gibberellin and zeatin) but salt tolerant PGPR are capable of maintaining and promoting the phytohormone balance in plants.
Auxin level which is important for plant growth and physiology has been reported to decline under salinity because of its antagonistic behavior with Abscisic acid (ABA). Studies in recent years revealed the importance of IAA and its positive effects on plants. The IAA produced by PGPR under natural or stressed conditions helps the plants in improving growth and yield. Among the Pseudomonas genera, strains Pseudomonas fluorescens, Pseudomonas chlororaphis, Pseudomonas putida, Pseudomonas extremoientalis have the potential of auxin production. Among Bacillus genus, Bacillus subtilis, Bacillus amyloliquefaciens have been reported to improve plant growth by improving auxin production in treated plants under salt stress.

Bacillus spp, Bacillus tequilensis, Bacillus subtilis were found to be effective in IAA production under natural conditions. The IAA producing Pseudomonas spp, Pseudomonas putida, Pseudomonas fluorescens have also been screened out previously.

L-tryptophan, the precursor of IAA, is naturally present in root exudates of plants. It is also synthesized by hydrolysis of proteins of dead cells, and is converted into indole acetic acid by the activity of plant growth promoting rhizobacteria. The leaf litter and dead decaying plant material are sources of proteins and amino acids like tryptophan which mostly occurs under salt stress. Previous hypothesis was based on the assumption that ABA is stress hormone but recent theory is based on production of indole acetic acid (IAA) is important for improving salt tolerance. The mechanism of salt tolerance induced by tryptophan has been documented by few researchers.

L-tryptophan application on plants has been demonstrated for the better growth of wheat, Philodendron erubescens and Zea mays by focusing its role as precursor of IAA. Several studies reveal the increasing potential of PGPR to produce higher IAA in culture media in the presence of L-tryptophan.
The plant growth and physiology has been affected by exogenous application of IAA or 
tryptophan. The previous findings approve the role of tryptophan in modulating protein, 
chlorophyll, sugar and amino acids. Plants are physiologically affected by PGPR application, 
and crop yield is enhanced by many folds owing to increased phytohormones production, and 
promotion in antioxidant activities under salt stress.

Application of L-tryptophan with PGPR in soil has proved very fruitful for increasing growth 
of different plants under natural and under salt stress. Application of L-tryptophan imparted 
positive effects on many crops like chickpea (Abbas et al., 2013) and wheat (Mohite, 2013). 
The growth and productivity of wheat has also been affected by the combination of PGPR 
and L-tryptophan under salt stress.

The role of IAA in salinity alleviation has also been demonstrated in sunflower and 
conversion of tryptophan to IAA by PGPR involved five different pathways, starting from 
tryptophan. It was evident in some microbes that IAA production was not terminated even 
when single pathway was blocked.

The pathogenic bacteria follows indole-3-acetamide pathway. In this pathway, tryptophan 
monooxygenase converts tryptophan to IAM and then IAM is converted into IAA. Bacteria 
like Agrobacterium tumefaciens, P. savastanoi, Pseudomonas syringae and Pantoea 
agglomerans follows this pathway.

P. agglomerans, Azospirillum, Bacillus, Bradyrhizobium, Enterobacter cloacae, 
Paenibacillus, Pseudomonas, Rhizobium and cyanobacteria followed a different pathway in 
which tryptophan is transaminated to indole-3-pyruvic acid (IPA) which is decarboxylated to 
indole-3-acetalde-hyde (IAAld) and finally oxidized to IAA.
In the tryptamine pathway, tryptamine is decarboxylated into tryptophan which is converted to IAM by an amine oxidase. In *Agrobacterium* and *Rhizobium* spp, indole-3-acetonitrile (IAN) is converted into IAM which is finally converted into IAA. Microbial strains having ability to produce higher and lower amount of IAA, or indole acetamide (IAM) have resulted in increased growth and yield of wheat.

Auxin biosynthesis in bacteria is affected by a number of factors including environmental stress, pH, osmotic and matrix stress, carbon starvation, and the composition of the root exudates. Microbial bioinoculants have ability to improve available nutrients in most of soils.

*Bacillus cereus* has the potential to increase the yield, growth and nutrition of broccoli plant under organic farming, it is efficient phosphate solubilizer and a biopesticide against the fungal pathogens that attack the plants during the nodulation stage of Pigeon Pea.

*Pseudomonas fluorescenc*e in consortium with other microbial strains improved growth and yield of chickpea and increase roots and shoots mass in sugarcane. *Pseudomonas putida* has been used for promoting growth of maize. *Pseudomonas* inoculation on winter wheat depends upon development phase of wheat as well as on population size of *Pseudomonas*.

The plant hormone ABA, is an important signal for the physiological and molecular responses to water-limiting stresses, such as desiccation, salt stress, and cold (Golldack et al., 2014). Under natural conditions, a negative correlation exists between growth and ABA level in plants but under stress the mechanism is reversed.

3.1. Materials and Methods

3.1.1. Plant material and growing conditions

Two isolates *Pseudomonas moraviensis* (accession No. LN714047) and *Bacillus cereus* (accession No. LN714048) from rhizosphere soil of halophytic herbs *Chrysopogon aucheri*
and *Cenchrus ciliaris* respectively were applied on wheat. Treatments include (1) single inoculation of *Pseudomonas moraviensis*, (2) single inoculation of *Bacillus cereus*, (3) inoculation of *Pseudomonas moraviensis* with tryptophan, (4) inoculation of *Bacillus cereus* with tryptophan and (5) tryptophan addition without any PGPR. Un-inoculated plants were taken as control. Plants sampling was done at early vegetative stage (57 days after sowing) for physiological parameters and at maturity for yield parameters.

Prior to sowing seeds were surface sterilized with 70% ethanol for 5 min followed by soaking the seeds in 10% chlorox for 2-3 min and successively washed with autoclaved distilled water. The sterilized seeds were soaked for 30 min in 7d old bacterial culture having $10^6$ cells/ml. After shade drying for 15 min seeds were sown.

### 3.1.2. Field experiment

The field experiment was conducted for two consecutive years i.e 2010 and 2011. Seeds were sown in saline sodic soil ($EC = 4.76 \, \text{dSm}^{-1}$) of field measuring 10x10m$^2$ at Soil Salinity Research Institute Pindibhattian. Seeds were also planted in the unstresseded soil of field ($EC = 0.43 \, \text{dSm}^{-1}$) at Quaid-e- Azam University Islamabad. Distance between two rows was 36cm. The RCBD design was used in the field. L-tryptophan at 0.21 mg/L ($10^{-5} \text{M}$) was applied with irrigation water in rooting zone of seedlings after 7 d of seed germination. Plants sampling was done at early vegetative stage (57 days after sowing) for physiological parameters and at maturity for yield parameters.

### 3.1.3. Pots experiment

Pots experiment was conducted at Quaid-e-Azam University Islamabad for two consecutive years i.e 2010 and 2011. Earthen pots measuring 17x20cm$^2$ were filled with garden soil which was autoclaved twice and mixed with sand in 3:1 ratio. Each pot was filled with 8Kg
sand soil mixture in (3:1) and no chemical or organic fertilizer was added. In each pot, 10-15 seeds were sown and were thinned to 5 plants/pot. Salinity was induced by adding 150 mM NaCl (EC= 3.6 dSm\(^{-1}\)), after 3d of seed germination. Similar to field plants, L- tryptophan at 0.21 mg/L (10\(^{-5}\)M) was applied with irrigation water in rooting zone of seedlings after 7 d of seed germination.

3.2. Results

3.2.1. Survival efficiency of PGPR strains in rhizosphere soil filtrate from saline sodic soil

The survival efficiency of *Pseudomonas moraviensis* and *Bacillus cereus* as measured by colony forming units (cfu) (Fig 3.1) showed linear decrease with increasing salt concentration in rhizosphere soil filtrate. The cfu of *Pseudomonas moraviensis* and *Bacillus cereus* was lower in the mixture of salts. *Bacillus cereus* was relatively less tolerant to salts mixture (NaCl, Na\(_2\)CO\(_3\) and NaHCO\(_3\)) present in the saline soil of the field, at 2 dSm\(^{-1}\) (Fig 3.1) showing 87% decrease over control. The decrease in CFU of *Pseudomonas moraviensis* was 82% at same EC.

3.2.2. Survival efficiency of PGPR strains in NaCl

A gradual decline in cfu was recorded for all PGPR, with increasing NaCl concentration in culture media (Fig 3.2). At highest concentration of NaCl provided (4.7 dS m\(^{-1}\)), the decline in cfu of *Bacillus cereus* and *Pseudomonas moraviensis* was 66% and 57% respectively over control (at 0 dS m\(^{-1}\)).

3.2.3. Effects of tryptophan addition in PGPR culture

Addition of tryptophan to the culture media increased the growth of PGPR as compared to control measured as O.D at 660 nm (Fig 3.3). The maximum growth of PGPR isolates was at
0.21 mg/l of tryptophan where the O.D of Bacillus cereus and Pseudomonas moraviensis were 64% and 54% higher respectively over control in which tryptophan was added. The PGPR showed gradual decrease in OD when 2.1, 21 and 210 mg/L tryptophan was added in cultures.

3.2.4. Survival efficiency of PGPR in soil

Colony forming unit (cfu) (Fig 3.4) of Pseudomonas moraviensis was 20% higher in rhizosphere soil of pots grown wheat, at 57 DAS as compared to Bacillus cereus. Addition of tryptophan to the soil further increased the cfu of Pseudomonas moraviensis and Bacillus cereus by 4-9% as compared to that of without tryptophan addition.

3.2.5. Effects of PGPR inoculation with or without tryptophan on physicochemical properties of soil in potted plants grown under salt stress

In pot experiment during 2010-2011, the PGPR decreased the electrical conductivity, pH and sodium absorption ratio (SAR) (Table 3.3), which were further reduced by the addition of 0.21 mg/l tryptophan in rooting zone. The inoculation of Pseudomonas moraviensis in soil decreased the SAR by 33% over control and tryptophan addition at 0.21 mg/L further decreased 20% SAR of rhizosphere soil. Decrease in SAR was 40% following the Bacillus cereus inoculation which was further decreased by 10% when tryptophan was added. The significant decrease 29% in electrical conductivity (EC) was observed when tryptophan was added with Bacillus cereus.

The single inoculation of PGPR increased organic matter by 13% over control. Addition of tryptophan with Bacillus cereus and Pseudomonas moraviensis improved organic matter significantly by 30% and 23% respectively. Similar trend was observed in 2011-2012.
3.2.6. Effects of PGPR inoculation with or without tryptophan on physicochemical properties of soil of saline sodic field

In 2010-2011 under saline sodic field, both *Pseudomonas moraviensis* and *Bacillus cereus* inoculation showed no significant decrease in the pH and SAR of the rhizosphere soil (Table 3.4). The decrease in SAR was 40% greater in potted plants than that of the field.

The EC of treated soil was significantly different in all the treatments except tryptophan treatment made to un-inoculated control plants. *Bacillus cereus* being most effective and showed 20% decrease in EC, which was further decreased by 8% in the presence of tryptophan. *Pseudomonas moraviensis* decreased 16% EC of soil which was further decreased by 9% in presence of tryptophan.

Organic matter of rhizosphere soil treated with PGPR and tryptophan combination was significantly higher (Table 3.4). Increase in soil organic matter was 66% over control when *Bacillus cereus* was added with tryptophan. This increase was 45% when *Pseudomonas moraviensis* was added with tryptophan.

The tryptophan addition with PGPR resulted in greater (20%) decrease in EC of the rhizosphere soil of field grown plants over the rhizosphere soil of potted plants (Table 3.3). The increase in organic matter was higher only when PGPR were inoculated with tryptophan and magnitude of increase was 25% greater in field grown plants.

3.2.7. Effects of PGPR inoculation with or without tryptophan on soil nutrient status of potted plants grown under salt stress

During 2010-2011, PGPR increased the availability of P and enriched the rhizosphere with NO$_3^-$–N in pots grown plants under salt stress (Table 3.5). Maximum increases in NO$_3^-$–N were 47% and 37% due to *Pseudomonas moraviensis* and *Bacillus cereus* respectively which
were further augmented (10-20%) by tryptophan addition. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan increased NO$_3$–N by 20-24% in potted plants treated with NaCl over potted plants grown under unstressed condition.

Increase in P content of the soil (Table 3.5) was 16% and 32% following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* in soil further enhanced 50% and 70% P.

The increase in P contents of potted plant was 35% higher than field grown plant when tryptophan was added with PGPR. Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* and tryptophan addition increased P content of soil by 12% and 35% respectively in potted plants grown under stressed condition over potted plants grown under unstressed condition (Table 3.9).

The application of *Pseudomonas moraviensis* and *Bacillus cereus* improved K$^+$ content (Table 3.5) by 27% and 36% while tryptophan addition with these PGPR further enhanced 30% K$^+$ content in the soil. The inoculation of PGPR with or without tryptophan exhibited 10% greater increase in K$^+$ content of potted plants than that of field grown plants. In potted plants grown under stressed condition, single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased P content of soil by 15% (Table 3.9) while tryptophan addition with PGPR increase 35% P over potted plants grown under unstressed condition.

The Ca$^+$ content (Table 3.5) of the soil was 17% higher when *Pseudomonas moraviensis* and *Bacillus cereus* were applied singly. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased Ca content by 10%.

The increase in Mg content of soil (Table 3.5) was 60% higher when *Pseudomonas moraviensis* and *Bacillus cereus* were applied singly. Tryptophan addition with *Pseudomonas*
moraviensis further increased Mg content by 30%. Similarly Bacillus cereus application with tryptophan showed 45% greater Mg over single inoculation. Potted plants grown under stressed condition showed 20% greater Mg in single inoculation over field grown plants (Table 3.7). The magnitude of increase was 35% higher when tryptophan was added with PGPR.

The PGPR addition with tryptophan induced 13% decrease in Na⁺ content (Table 3.5) of soil. This decrease was 20% when Bacillus cereus with tryptophan was applied and 16% when Pseudomonas moraviensis with tryptophan was applied. Similarly the added tryptophan with Pseudomonas moraviensis and Bacillus cereus decreased soil Cl by 27% and 35% respectively over control. Similar trend was observed during 2011-2012 (Table 3.6).

3.2.8. Effects of PGPR inoculation with or without tryptophan on soil nutrient status of saline sodic field

In 2010-2011 under saline sodic field condition, inoculation of Pseudomonas moraviensis and Bacillus cereus increased NO₃-N contents (Table 3.7) of rhizosphere soil by 25-27%. Addition of tryptophan with Pseudomonas moraviensis and Bacillus cereus further increased 10% and 30% NO₃-N contents respectively over control. PGPR inoculation with tryptophan in potted plants showed 15% greater increase in soil NO⁻₃ than field.

The increase in P contents of rhizosphere soil treated with Pseudomonas moraviensis and Bacillus cereus with tryptophan were 21% and 25% higher over control (Table 3.7). Increase in P content was 15% higher in saline sodic field soil over unstressed field soil following the inoculation of Pseudomonas moraviensis and Bacillus cereus. Similarly, tryptophan addition with Pseudomonas moraviensis and Bacillus cereus increased P content by 22% in saline sodic field.
The increase in $K^+$ content of soil (Table 3.7) was 40% and 37% following the application of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with both PGPR resulted in further 15% increase in K. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* increased K content by 20% in saline sodic field over unstressed field (Table 3.11).

The $Ca^+$ content (Table 3.7) were improved by 33% and 25% following the application of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan respectively. Inoculation of PGPR with or without tryptophan showed equal magnitude of increase (30%) in Ca content, both in potted and field grown plant. The inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* equally increased Ca content of saline sodic and unstressed field however tryptophan addition with PGPR resulted 16% increase in Ca content of unstressed field soil (Table 3.11).

Increase in Mg content (Table 3.7), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* was 50% over control. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased Mg contents by 22% and 15% respectively.

The decrease in Na content (Table 3.7) was significant i.e 33% and 22% decreases when *Pseudomonas moraviensis* and *Bacillus cereus* were applied with tryptophan respectively. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan also decreased Cl content by 67% and 55% respectively over control. The magnitude of decrease in Na and Cl was 13% and 40% greater in field grown plants over potted plants. Similar trend was observed in 2011-2012 (Table 3.8).

3.2.9. Effects of PGPR and tryptophan application on nutrient status of rhizosphere soil of potted plants grown under unstressed condition
In potted plants under unstressed condition during 2010-2011, *Pseudomonas moraviensis* increased the soil organic matter (Table 3.9 and Table 3.10) by 37% and tryptophan addition further increased organic matter by 60% over control. Similarly, inoculation of *Bacillus cereus* increased organic matter of rhizosphere soil by 68% and a further 40% increase was observed when tryptophan was added with *Bacillus cereus*.

Increase in organic matter was 60% higher in potted plants as compared to field grown plants. The soil organic matter of potted plants grown under unstressed condition was 24% and 50% greater over the organic matter of potted plants treated with NaCl, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* resulted in 70% greater organic matter in potted plants grown under unstressed condition over potted plants treated with NaCl (Table 3.5).

Inoculation of PGPR increased the phosphorous availability and enriched the rhizophere with NO$_3$–N in field grown plants under unstressed condition. Maximum increase in NO$_3$–N (Table 3.9, Table 3.10) was 24% and 27% due to *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with two PGPR resulted further 10% increase in P and NO$_3$–N of pots and field grown plants.

Increase in phosphorus content of soil was 20% over control when PGPR were inoculated singly. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* augmented P content by 10%. Both potted and field grown plants treated with PGPR, with or without tryptophan contributed equally in improving soil NO$_3$-N and P.

*Pseudomonas moraviensis* and *Bacillus Cereus* inoculation with tryptophan improved K content equally by 20% over control. Increase in Ca$^+$ content was 25% and 16% following the inoculation of *Pseudomonas moraviensis* and *Bacillus Cereus* respectively. Tryptophan
addition with *Pseudomonas moraviensis* further increased Ca content by 15% and *Bacillus Cereus* with tryptophan by 24%. Inoculation of PGPR with or without tryptophan in potted plants enhanced 10% Ca content over field grown plant (Table 3.11).

Inoculation of *Pseudomonas moraviensis* and *Bacillus Cereus* enhanced Mg⁺ content by 45% and 60% respectively. Addition of tryptophan with *Pseudomonas moraviensis* further enhanced Mg content by 60%. The maximum increase (171%) over control was observed when *Bacillus Cereus* was added with tryptophan. Increased in Mg was 30% greater in potted plants over field grown when PGPR were inoculated singly. This increase was even greater (70-90%) when tryptophan was added with PGPR. Similar trend was observed during 2011-2012 in potted plants (Table 3.10).
3.2.10. Effects of PGPR and tryptophan application on nutrient status of rhizosphere soil under unstressed field

During 2010-2011 under unstressed condition, soil organic matter in the rhizosphere of field grown plants was significantly (38 - 42%) higher when two PGPR were inoculated with tryptophan at 0.21 mg/L under field condition (Table 3.11). Increase in organic matter of unstressed field soil was 6% over saline sodic field, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Tryptophan addition with PGPR in unstressed soil had 10% greater organic matter over saline sodic field (Table 3.7).

Inoculation of PGPR increased the NO$_3$–N in field grown plants by 18%. Maximum increase in NO$_3$–N (Table 3.11, Table 3.12) was 27% and 32% over control when *Pseudomonas moraviensis* and *Bacillus cereus* were applied with tryptophan. Increase in phosphorus content of soil was 26% over control when PGPR were inoculated singly. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* further augmented P content by 10% and 15% respectively. Increase in NO$_3$–N of unstressed field soil was 4-10% over saline sodic field (Table 3.7), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan.

The K content of rhizosphere soil (Table 3.11) were 34% and 21% higher over control only when *Pseudomonas moraviensis* and *Bacillus cereus* were added with tryptophan respectively. Increase in K content of field grown plants was 14% higher over potted plant.

Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* resulted 18% greater Ca over control while tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* further resulted 24% and 8% increase in Ca respectively. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan increased Ca content by 10% in potted plants grown under unstressed condition over potted plants treated with NaCl (Table
3.5. *Pseudomonas moraviensis* and *Bacillus Cereus* inoculation with tryptophan improved Mg content (Table 3.11) by 24% and 28% respectively over control. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus Cereus* further increased Mg content by 14% and 10% respectively.

Similar trend was observed during 2011-2012 in pots and field grown plants (Table 3.12). Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan increased Mg content by 15-17% in potted plants grown under unstressed condition over potted plants treated with NaCl (Table 3.5).

**3.2.11. Effects of PGPR with or without tryptophan on nutrients accumulation of leaves of potted plants grown under salt stress**

In potted plants under salt stress during 2010-2011, the accumulation of NO$_3$-N was 30% higher over control when *Pseudomonas moraviensis* and *Bacillus Cereus* were applied with tryptophan (Table 3.13). Similarly P accumulation was increased by 30% following the application of *Pseudomonas moraviensis* and *Bacillus Cereus* added with tryptophan.

*Pseudomonas moraviensis* and *Bacillus Cereus* enhanced the K by 22% and 17% respectively. Addition of tryptophan to PGPR resulted 30% increase in K over control.

Ca$^+$ accumulation in leaves treated with *Pseudomonas moraviensis* and *Bacillus Cereus* were 33% and 16% greater over control. This effect was further augmented by the addition of tryptophan and *Bacillus Cereus* showed 50% greater Ca contents as compared to single inoculation.

The increase in Mg accumulation in inoculated leaves (Table 3.13) was 21% over control. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus Cereus* further increased 28% and 17% greater Mg. Similarly Cl content of leaves was decreased by 35% over control.
only when *Pseudomonas moraviensis* and *Bacillus Cereus* were applied with tryptophan. Similar trend was observed during 2011-2012 (Table 3.14).

The Na accumulation in leaves of plants treated with *Pseudomonas moraviensis* and *Bacillus Cereus* was 30% lower over control. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus Cereus* further decreased the Na accumulation by 25%.

### 3.2.12. Effects of PGPR with or without tryptophan on nutrients accumulation of leaves of plants grown in saline sodic field

During 2010-2011 the plants grown under saline sodic field showed 18% and 25% decreases in Na accumulation in leaves (Table 3.15) following the application of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan respectively. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* without tryptophan showed 5% greater Na in field grown plants over potted plants (Table 3.13).

The inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased K⁺ content by 27% and 38% respectively while addition of tryptophan with these PGPR further enhanced K accumulation by 25% (Table 3.15). Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* without tryptophan showed 15-25% greater K in field grown plants over potted plants. Increase in K content of saline sodic field over unstressed field was 12% and 8% following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with PGPR accumulated 9% higher K content in the leaves grown under saline sodic field (Table 3.15).

Ca⁺ content of leaves were 28% higher when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated singly, while addition of tryptophan with *Bacillus Cereus* and *Pseudomonas moraviensis* further increase Ca⁺ contents by 45% and 57% respectively over control (Table
3.15). Increase in Ca contents of leaves of field grown plants was 5-11% higher in single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Tryptophan addition with *Pseudomonas moraviensis* accumulated 18% greater Ca content in leaves of field grown plants, while *Bacillus cereus* application with tryptophan resulted equal increase both in potted and field grown plants over control. Increase in Ca content of saline sodic field grown plants was 10% and 8% over unstressed field grown plants (Table 3.17), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with *Bacillus cereus* accumulated 18% greater Ca content in leaves of field grown plants over control. Increase in Ca content of saline sodic field grown plants was 10% and 8% over unstressed field grown plants (Table 3.17), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Tryptophan addition with *Bacillus cereus* accumulated 7% higher K content in leaves grown under saline sodic field.

Similarly, Mg$^+$ contents (Table 3.15) were 27% higher when *Pseudomonas moraviensis* was used as bioinoculant and tryptophan addition with *Pseudomonas moraviensis* further increased 6% Mg$^+$ in leaves. *Bacillus cereus* enhanced Mg contents by 44% over control, only when tryptophan was added. The single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* exhibited 5-10% greater Mg in field grown plants over potted plants. Addition of tryptophan with PGPR resulted 10-15% higher Mg in field grown plants over potted plants (Table 3.13). Similar trend was observed during 2011-2012 in pots and field grown plants.

**3.2.13. Effects of PGPR and tryptophan application on nutrient status of leaves of potted plants grown under unstressed conditions**

During 2010-2011 in potted plant under unstressed condition, *Pseudomonas moraviensis* and *Bacillus cereus* improved the NO$_3$-N acquisition in treated wheat leaves by 70% (Table 3.16). Tryptophan addition with *Pseudomonas moraviensis* further increased NO$_3$-N by 88%. Similarly, *Bacillus cereus* in the presence of tryptophan resulted in further 50% increase in NO$_3$-N. Leaves of potted plants grown under unstressed condition accumulated 60% higher
General Introduction

NO$_3$-N over potted plants treated with NaCl (Table 3.13). Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* increased NO$_3$-N by 30% and 40% respectively in potted plants grown under unstressed condition (Table 3.16).

The accumulation of P was 53% higher over control, in the leaves treated with *Pseudomonas moraviensis* (Table 3.16). Addition of tryptophan with *Pseudomonas moraviensis* further increased P contents by 12%. *Bacillus cereus* increased P contents by 44% and tryptophan addition further increased P content by 19%.

The P content was 25-30% higher in potted plants over field grown plant (Table 3.17) when *Pseudomonas moraviensis* and *Bacillus cereus* were inoculated in the presence or absence of tryptophan. The inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan increased P content by 25% in potted plants grown under unstressed condition over potted plants treated with NaCl (Table 3.13).

The Ca contents of leaves (Table 3.16) were increased by 28% in pots grown plants, following the treatment of *Pseudomonas moraviensis* and *Bacillus cereus*. Tryptophan addition further enhanced Ca by 37%. *Bacillus cereus* inoculation was equally affective in potted plants grown under stressed or unstressed condition. *Pseudomonas moraviensis* inoculation accumulated 14% higher Ca in the leaves of potted plants grown under unstressed condition over potted plants treated with NaCl (Table 3.13). Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* increased Ca accumulation by 30% and 9% respectively in potted plants grown under unstressed condition.

Similarly the K contents of leaves (Table 3.16) were increased significantly (30%), and tryptophan addition further increased K contents by 15-20%. The Mg contents were increased by 30-40% when PGPR were applied singly, and with tryptophan further 15-20% higher Mg was observed. The inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or
without tryptophan increased Mg content by 20% in potted plants grown under unstressed condition over potted plants treated with NaCl (Table 3.13).

*Bacillus cereus* and *Pseudomonas moraviensis* inoculation increased Mg content of potted plants grown under unstressed condition by 9% and 19% respectively (Table 3.16) over potted plants treated with NaCl (Table 3.13). Addition of tryptophan with *Bacillus cereus* increased Mg accumulation by 20% in potted plants grown under unstressed condition (Table 3.16).

**3.2.14. Effects of PGPR with or without tryptophan on nutrients accumulation of leaves of field grown plants grown under unstressed condition**

During 2010-2011, under unstressed field condition, NO$_3$-N and P contents (Table 3.17) of leaves were 35% and 23% higher over control, when *Pseudomonas moraviensis* was applied. Addition of tryptophan with *Pseudomonas moraviensis* further increased NO$_3$-N and P contents by 97% and 40%. *Bacillus cereus* increased NO$_3$-N contents by 38% and tryptophan addition further increased NO$_3$-N contents by 75%. The P contents were increased by 68% when tryptophan was added with *Bacillus cereus*. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* without tryptophan showed 35% greater NO$_3$-N in field grown plants over potted plants. Similarly, addition of tryptophan with PGPR accumulated 15% NO$_3$-N in field grown plants.

The Ca$^+$ content of leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* were 20% higher over control. Addition of tryptophan with these PGPR further enhanced Ca$^+$ content by 35%. Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* without tryptophan showed 10% greater Ca in field grown plants over potted plants.
Increases in K\(^+\) contents of leaves were 15\% and 25\%, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further improved K accumulation by 20-30\%. Increase in K content was 10-15\% higher in the leaves of field grown plants over potted plants (Table 3.16), when PGPR were inoculated with or without tryptophan.

Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* exhibited 30\% greater Mg in leaves over control. *Pseudomonas moraviensis* + tryptophan further increased Mg\(^+\) contents of leaves by 38\%. Added tryptophan with *Bacillus cereus* further enhanced the Mg\(^+\) accumulation in leaves by 42\%. Similar trend was observed during 2011-2012 in potted plants. Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*, and addition of tryptophan with PGPR exhibited 10-20\% greater Mg in field grown plants over potted plants (Table 3.16). Increase in Mg content of unstressed field grown plants was 4\% over saline sodic field grown plants, following the inoculation of *Pseudomonas moraviensis*. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* accumulated 36\% and 31\% higher K content respectively in the leaves of plants grown under unstressed field.

at \((p = 0.05)\). Values represented in parentheses are standard error of mean

3.2.15. **Effects of PGPR inoculation with or without tryptophan on improving plant growth and physiology of potted plants grown under salt stress**

In potted plants under salt stress during 2010-2011, both *Pseudomonas moraviensis* and *Bacillus cereus* increased plant height (Table 3.18). Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased plant height by 29\% and 33\% respectively over control. The addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased plant height by 14\% and 8\% respectively.
Increase in plant height of potted plants under stressed or unstressed condition showed equal increase over control, following the inoculation of PGPR with or without tryptophan. However, *Bacillus cereus* inoculation with tryptophan showed 5% increase plant height in unstressed plants over stressed plants.

The *Pseudomonas moraviensis* increased the fresh weight (Table 3.18) by 16% and 18% over control respectively. Addition of tryptophan further increased the fresh weight by 45-50% in *Pseudomonas moraviensis* and *Bacillus cereus* inoculation respectively.

Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased chlorophyll contents by 8-16% in leaves under salt stress (Table 3.18). Addition of tryptophan with *Pseudomonas moraviensis* showed 13% increase. Similar trend was observed during 2011-2012 (Table 3.19).

Sugar contents (Table 3.18) were 8% and 19% higher over control when treated with *Pseudomonas moraviensis* and *Bacillus cereus* respectively under salt stress. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased sugar contents by 56% and 59% over control.

*Pseudomonas moraviensis* and *Bacillus cereus* increased proline content by 50% over control (Table 3.18). Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased the proline contents of leaves by 10-15%. Increase in proline content of potted plants under stressed condition was 19% and 39% higher over potted plants grown under unstressed condition, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*.

*Pseudomonas moraviensis* exhibited 60% higher SOD and 62% higher POD activities under salt stress (Table 3.18). *Bacillus cereus* exhibited 67% higher SOD and 96% higher POD
activities. In the presence of tryptophan, *Pseudomonas moraviensis* showed further 58% and 55% increases in SOD and POD activities respectively. Similarly, addition of tryptophan with *Bacillus cereus* exhibited further 73% higher SOD and 38% higher POD activities. Increase in POD activity was 20-30% greater in potted plants when PGPR were applied with or without tryptophan, over field grown plants (Table 3.20).

Increase in SOD activity was 33% and 49% in potted plants grown under stressed condition over potted plants grown under unstressed condition (Table 3.26) following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Similarly tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* enhanced 25% and 80% SOD activities in potted plants grown under stressed condition.

The POD activity was increased by 34% and 70% in potted plants grown under stressed condition over potted plants grown under unstressed condition (Table 3.26), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Similarly tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* enhanced 40% and 25% POD activities in potted plants grown under stressed condition. Similar trend was observed for plant height, fresh weight, Chlorophyll, sugar, proline, SOD and POD during 2011-2012 (Table 3.19).

### 3.2.16. Effects of PGPR inoculation with or without tryptophan on IAA, GA and ABA production of potted plants grown under salt stress

In potted plants during 2010-2011, rhizosphere soil of wheat inoculated with *Pseudomonas moraviensis* and *Bacillus cereus* contained 50% and 89% higher IAA respectively over uninoculated control (Table 3.20). Tryptophan addition with *Pseudomonas moraviensis* increased IAA content by 2 fold in rhizosphere soil. The increases in IAA content of leaves were 36% over control when plants were inoculated with PGPR. *Pseudomonas moraviensis* and *Bacillus cereus* in the presence of tryptophan exhibited 55% higher IAA over control in leaves.
In potted plants during 2010-2011, *Bacillus cereus* produced 6-8% higher gibberellic acid (Table 3.20) than that of *Pseudomonas moraviensis* in the soil. The plants treated with *Pseudomonas moraviensis* and *Bacillus cereus* had 35% and 29% higher GA contents over control in leaves. The rhizosphere soil inoculated with PGPR exhibited 24-31% GA over uninoculated control. Tryptophan addition resulted 50% higher GA in rhizosphere soil, and 62% higher in leaves. Similar trend was observed during 2011-2012.

In potted plants during 2010-2011, the *Bacillus cereus* produced higher ABA than *Pseudomonas moraviensis*. Addition of tryptophan to rhizosphere soil significantly augmented ABA production both in rhizosphere soil and plant leaves. The ABA contents (Table 3.20) of leaves of inoculated plants were 13-16% higher than that of IAA. Similarly, the *Bacillus cereus* induced 30% higher ABA in the rhizosphere soil than that of uninoculated control. As compared to un-inoculated control, ABA contents of the inoculated leaves were 80-84% higher when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated separately. Addition of tryptophan to plants further increased the ABA content of soil by 30-50% whereas in leaves 12-15% higher ABA was observed over un-inoculated control. Similar trend was observed during 2011-2012.

3.2.17. Effects of PGPR inoculation with or without tryptophan on improving plant growth and physiology of field grown plants grown under saline sodic condition

During 2010-2011 under saline sodic field condition (Table 3.21), increase in plant height was 26% and 29% following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* resulted further 11% and 14% increase in plant height respectively. Plants height of field grown plants was 7% greater over potted plants (Table 3.18) when *Pseudomonas moraviensis* and *Bacillus cereus* were applied with or without tryptophan.
*Pseudomonas moraviensis* and *Bacillus cereus* increased the fresh weight (Table 3.21) of plants by 41% and 89% over control and further 6-10% increase was observed when tryptophan was added with these PGPR. Fresh weight of field grown plants was 35% greater over potted plants (Table 3.18) when *Pseudomonas moraviensis* and *Bacillus cereus* were applied with or without tryptophan.

Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased chlorophyll contents of leaves (Table 3.21) by 21-25% and tryptophan addition resulted further 6-20% increase. The increase in chlorophyll content of field grown plants was 10% higher over potted plants (Table 3.18) following the application of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan.

The proline content (Table 3.21) was 91% and 49% greater over control in *Pseudomonas moraviensis* and *Bacillus cereus* inoculation. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased the proline contents by 30% and 98% over control respectively. The proline content of field grown plants was 40% and 11% higher over potted plants (Table 3.18) when *Pseudomonas moraviensis* and *Bacillus cereus* were applied without tryptophan. The increase in chlorophyll content of field grown plants was 60% and 82% higher over potted plants following the application of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan. Increase in proline content in the leaves of saline sodic field grown plants was 74% and 39% over unstressed field grown plants (Table 3.27), following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Plants grown under saline sodic field condition had 93% and 123% higher proline when *Pseudomonas moraviensis* and *Bacillus cereus* were applied with tryptophan.

Antioxidant (superoxide dismutase (SOD) and peroxidase (POD) activities) (Table 3.21) were 66% and 34% higher in plants inoculated with *Pseudomonas moraviensis* while *Bacillus*
cereus showed 86% and 65% higher SOD and POD activities respectively. Addition of tryptophan with Pseudomonas moraviensis and Bacillus cereus further increased 20% and 40% SOD respectively. Similarly, POD activities were further increased by 60% following the inoculation of Pseudomonas moraviensis and Bacillus cereus with tryptophan. The increase in SOD activity of field grown plants was 20% higher over potted plants (Table 3.18) following the application of Bacillus cereus. However, addition of tryptophan with Pseudomonas moraviensis and Bacillus cereus was more beneficiary for potted plants and increased SOD activity by 18-24% over field grown plants (Table 3.21). The increase in POD activity of potted plants was 30% and 82% higher over potted plants following the application of Pseudomonas moraviensis and Bacillus cereus with tryptophan.

SOD activity of leaves of saline sodic field grown plants (Table 3.21) was 26% and 30% over unstressed field grown plants, following the inoculation of Pseudomonas moraviensis and Bacillus cereus. Plants grown under saline sodic field condition had 36% and 40% higher SOD activity when Pseudomonas moraviensis and Bacillus cereus were applied with tryptophan.

Increase in POD activity in the leaves of saline sodic field grown plants (Table 3.21) was 25% and 30% over unstressed field grown plants (Table 3.27), following the inoculation of Pseudomonas moraviensis and Bacillus cereus. Plants grown under saline sodic field condition had 37% and 50% higher POD activity when Pseudomonas moraviensis and Bacillus cereus were applied with tryptophan.

3.2.18. Effects of PGPR inoculation with or without tryptophan on IAA, GA and ABA production of saline sodic field grown plants

During 2010-2011 under saline sodic field condition, Bacillus cereus produced 67% more IAA in the culture as compared to that of Pseudomonas moraviensis (Table 3.22). The
rhizosphere soil of wheat inoculated with *Pseudomonas moraviensis* and *Bacillus cereus* exhibited 50% and 89% higher IAA respectively over control. Similarly, the plant leaves treated with PGPR contained 20-22% higher IAA over control. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* increased IAA contents by 107% and 117% in the rhizosphere soil. This increase was 30-40% higher in leaves. Similar trend was observed during 2011-2012.

During 2010-2011 under saline sodic field condition, *Pseudomonas moraviensis* produced 18% more gibberellic acid (Table 3.23) than *Bacillus cereus*. *Bacillus cereus* and *Pseudomonas moraviensis* exhibited 57% and 39% higher GA in leaves. This increase was 12-19% higher in rhizosphere soil. Tryptophan addition with PGPR resulted 27% and 81% greater GA in rhizosphere soil and leaves respectively over un-inoculated plants. Similar trend was observed during 2011-2012.

During 2010-2011 under saline sodic field condition, the *Bacillus cereus* produced higher ABA than that of *Pseudomonas moraviensis*. Tryptophan addition to rhizosphere soil significantly improved ABA production in rhizosphere soil and plant leaves. The ABA content (Table 3.24) of PGPR isolates as well as leaves of inoculated plants was 50% less than that of IAA. The ABA contents of *Pseudomonas moraviensis* were 107% higher in rhizosphere soil than that of un-inoculated control. As compared to un-inoculated control, the ABA contents in the leaves were 57% and 45% higher in *Pseudomonas moraviensis* and *Bacillus cereus* respectively. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* increased the ABA content of soil by 131% and 124% respectively. The leaves treated with *Pseudomonas moraviensis* and *Bacillus cereus* and added tryptophan exhibited 65-78% more ABA over un-inoculated control. Similar trend was observed during 2011-2012.
3.2.19. Effects of PGPR inoculation with or without tryptophan on wheat growth of potted plants grown under unstressed condition

During 2010-2011 under unstressed condition, both *Pseudomonas moraviensis* and *Bacillus cereus* increased plant height (Table 3.25 and Table 3.26) in potted plants. Inoculation of *Pseudomonas moraviensis* increased plant height by 32% over control. Tryptophan addition with *Pseudomonas moraviensis* further increased plant height by 9%. Similarly, *Bacillus cereus* increased plant height by 31% and in the presence of tryptophan 4% further increase was observed. Increased in plant height was 5% higher in field grown plants (Table 3.27) following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan.

The *Pseudomonas moraviensis* and *Bacillus cereus* increased the fresh weight by 20% over control (Table 3.25 and Table 3.26). Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased 45% fresh weight in pots. Fresh weight of aerial parts was 7-12% higher in field grown plants (Table 3.27) following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan.

During 2010-2011 under unstressed condition, inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased chlorophyll contents (Table 3.25 and Table 3.26) by 21% over control. Chlorophyll content was 10% higher in field grown plants following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with tryptophan (Table 3.27).

*Pseudomonas moraviensis* and *Bacillus cereus* improved the proline content of leaves by 31% and 21% over control respectively (Table 3.25 and Table 3.26). Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased the proline by 8% and 12% respectively. Increased in proline content was 15% higher in potted plants over field grown plants (Table 3.27) following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan.
Pseudomonas moraviensis showed 29% and 23% higher SOD and POD (Table 3.25 and Table 3.26) activities over control respectively. In the presence of tryptophan, Pseudomonas moraviensis exhibited further 17% higher SOD and 46% higher POD activities. Bacillus cereus showed 11% higher SOD and 28% higher POD while addition of tryptophan exhibited further 22% higher SOD and 50% higher POD. Similar trend was observed for plant height, fresh weight, chlorophyll, proline, SOD and POD activities, during 2011-2012 (Table 3.25 and Table 3.26). Inoculation of Pseudomonas moraviensis and Bacillus cereus with or without tryptophan increased SOD activity equally in potted and field grown plants (Table 3.27) however increase in POD activity was 14% higher in field grown plants.

3.2.20. Effects of PGPR inoculation with or without tryptophan on plant growth and physiology of field grown plants grown under unstressed condition

During 2010-2011 under unstressed condition, inoculation of Pseudomonas moraviensis and Bacillus cereus increased plant height by 32% in field grown plants over control. Tryptophan addition with Pseudomonas moraviensis further increased plant height by 9%. Similarly, Bacillus cereus application with tryptophan further increased plant height by 7%. Increase in plant height of unstressed field grown plants was 6% over saline sodic field grown plants, following the inoculation of Pseudomonas moraviensis and Bacillus cereus.

Pseudomonas moraviensis inoculation increased the fresh weight by 18% (Table 3.27). Addition of tryptophan further increased 13% fresh weight in pots. The single inoculation of Bacillus cereus exhibited 11% greater fresh weight over control which was further increase by 28% when tryptophan was added. The fresh weight was increased by 5% in potted plants grown under unstressed condition over potted plants grown under stressed condition, following the inoculation of Pseudomonas moraviensis and Bacillus cereus. Inoculation of Pseudomonas moraviensis increased chlorophyll contents (Table 3.27) by 26% over control.
Addition of tryptophan with *Pseudomonas moraviensis* further increased chlorophyll contents by 12%. *Bacillus cereus* inoculation exhibited significantly higher (35%) chlorophyll content in leaves. Addition of tryptophan with *Bacillus cereus* further increased 11% chlorophyll. Chlorophyll content was increased by 8% in potted plants grown under unstressed condition over potted plants grown under stressed condition, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Addition of tryptophan with PGPR increased chlorophyll contents by 20% in potted plants grown under unstressed condition. Increase in chlorophyll content in the leaves of unstressed field grown plants was 5-10% over saline sodic field grown plants, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*.

Inoculation of *Pseudomonas moraviensis* improved the proline content of leaves by 17%. *Bacillus cereus* increased proline content (Table 3.27) by 12%. Addition of tryptophan with *Pseudomonas moraviensis* further increased the proline by 10%. Addition of tryptophan with *Bacillus cereus* further increased proline by 14%.

Plants treated with *Pseudomonas moraviensis* showed 20% higher SOD and 30% higher POD (Table 3.27) activities in field. In the presence of tryptophan *Pseudomonas moraviensis* exhibited further 14% higher SOD and 16% higher POD. *Bacillus cereus* showed 18% higher SOD and 36% higher POD. Addition of tryptophan exhibited further 27% higher SOD and 34% higher POD activities. Similar trend was observed for plant height, fresh weight, chlorophyll, proline, SOD and POD activities, during 2011-2012.

### 3.2.21. Effects of PGPR inoculation with or without tryptophan on wheat yield of potted plants grown under salt stress
In potted plants under salt stress during 2010-2011, *Pseudomonas moraviensis* and *Bacillus cereus* inoculation with tryptophan significantly (20%) increased spike length of wheat at maturity over control (Table 3.28).

*Pseudomonas moraviensis* and *Bacillus cereus* increased 16% and 25% seeds/spike respectively. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased (13%) seeds/spike.

Both *Pseudomonas moraviensis* and *Bacillus cereus* increased seed weight equally by 36% over control and addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* increased seed weight by 5%. Similar trend was observed during 2011-2012.

3.2.22. Effects of PGPR inoculation with or without tryptophan on wheat yield of saline sodic field grown plants

During 2010-2011 under saline sodic field condition, increase in number of plant/ m² (Table 3.29) was 29% over control when *Pseudomonas moraviensis* and *Bacillus cereus* were inoculated singly. Addition of tryptophan with these PGPR resulted further 28% increase in number of plant/ m².

Increase in spike length of the wheat following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* was 20% greater over control. Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* further enhanced the spike length by 9%. Application of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan showed 10% greater length of spike in field grown plants over potted plants.

*Pseudomonas moraviensis* increased seeds/spike (Table 3.27) by 33% and seed weight by 18% over control. *Bacillus cereus* increased seed/spike by 7% over control. Addition of tryptophan with *Pseudomonas moraviensis* further increased the seed / spike by 32% over
single inoculation. Application of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan showed 5-15% seeds/ pike in field grown plants over potted plants.

Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* resulted in 18% increase in seed weight over control. *Pseudomonas moraviensis* with added tryptophan further increased seed weight by 9%. Tryptophan addition with *Bacillus cereus* showed no significant improvement in seed weight over single inoculation. In field grown plants seeds weight was 7% greater over potted plants following the application of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan. Similar trend was observed during 2011-2012.

**3.2.23. Effects of PGPR inoculation with or without tryptophan on wheat yield of potted plants grown under unstressed condition**

During 2010-2011, inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased the spike length (Table 3.30) by 23% and tryptophan addition with both the PGPR enhanced spike length by 8%. Spike length was increased by 13% in potted plants grown under unstressed condition over potted plants grown under stressed condition, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus*. Addition of tryptophan with PGPR increased chlorophyll contents by 11% in potted plants grown under unstressed condition.

Increase in number of seeds / spike was 29% greater over control following the application of *Pseudomonas moraviensis* and *Bacillus cereus*. Addition of tryptophan with *Pseudomonas moraviensis* and *Bacillus cereus* further increased seed spike by 18% and 26% respectively. Increased in seed /spike of potted plants grown under unstressed condition was 13% and 5% over potted plants grown under stressed condition, following the inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* respectively.
Pseudomonas moraviensis and Bacillus cereus inoculation increased thousand seed weight by 15% over control. Addition of tryptophan with PGPR was not beneficial for improving seed weight. Similar trend was observed during 2011-2012 in pots and field grown plants. Increased in seed weight of potted plants grown under unstressed condition was 3% over potted plants grown under stressed condition, following the inoculation of Pseudomonas moraviensis and Bacillus cereus

3.2.24. Effects of PGPR inoculation with or without tryptophan on wheat yield of field grown plants grown under unstressed condition

During 2010-2011 under unstressed field condition, Pseudomonas moraviensis and Bacillus cereus exhibited 8-10% more plants/m² in pots and field grown plants (Table 3.31) over control. Tryptophan addition with Pseudomonas moraviensis and Bacillus cereus further increased 27% and 20% plants/m² over control respectively.

Inoculation of Pseudomonas moraviensis significantly increased spike length by 15-18%. Addition of tryptophan with Pseudomonas moraviensis further increased the spike length by 10-16%. Application of Pseudomonas moraviensis and Bacillus cereus with or without tryptophan showed 8% greater length of spike in field grown plants over potted plants.

Pseudomonas moraviensis and Bacillus cereus increased seeds/spike by 25% over control. Addition of tryptophan with Bacillus cereus further increased 14% spike length while Pseudomonas moraviensis application with tryptophan exhibited 15% greater seeds/spike. Field grown plants exhibited 10% greater seeds/spike over potted plants following the inoculation of PGPR with or without tryptophan.

Pseudomonas moraviensis and Bacillus cereus increased the seed weight by 28% over control. Tryptophan addition with Pseudomonas moraviensis and Bacillus cereus resulted no
significant effects on seed number. Application of *Pseudomonas moraviensis* and *Bacillus cereus* with or without tryptophan showed 5% greater number of seeds in field grown plants over potted plants.

### 3.2.18. Cost benefit ratio

The cost economic benefit ratio for the production of per hectare was 1.12 and 1.34 for wheat crop grown in saline sodic field and unstressed field respectively. These results indicate that L-tryptophan addition may increase the farmer’s benefits by 12% and 34% in salt stressed and unstressed conditions respectively.
3.3. Discussion

Among endospore forming bacteria *Bacillus* are important for their contribution in agriculture. Physiologically they are strong candidates for plant growth promotion due to endospore formation and multilayered cell wall composition. Secretion of peptide signals, peptide antibiotic and extra cellular enzymes also made them compatible.

*Pseudomonas* is considered as better root colonizer than *Bacillus* due to better survival and production of variety of plant growth promoting substances.

The application of the endophytes as plant growth promoting bacteria (PGPB) and their role in crop improvement is widely documented. *Bacillus cereus* and *Pseudomonas moraviensis* have been isolated and tested for their growth promoting potential.

Presence of tryptophan in culture media has strong involvement in improving IAA production. Most of the rhizobacteria and almost all PGPR strains respond positively to the presence of tryptophan and modulate the synthesis of IAA. Previously tryptophan addition in culture media with *Bacillus* and *Pseudomonas* strains augmented the IAA production.

The greater cfu of *Pseudomonas moraviensis* over *Bacillus cereus* is likely due to the utilization of various C/N substances than that of *Bacillus cereus* and higher phosphorus solubilizing ability of *Pseudomonas moraviensis*. *Pseudomonas* spp are considered as better PGPR for their better survival, motility and root colonization as compared to *Bacillus* spp. The increased growth in culture media supplemented with tryptophan is possibly mediated by tryptophan induced IAA production and IAA is involved in growth stimulation.

*Pseudomonas fluorescens, Pseudomonas chlororaphis, Pseudomonas putida, Pseudomonas extremoientalis* have the potential of auxin production. Similarly, *Bacillus subtilis, Bacillus
*Amyloliquefaciens* have been reported to improve plant growth by improving auxin production in treated plants under salt stress.

Application of PGPR with tryptophan improved soil organic matter. Previously IAA blended with nitrogen enriched compost as source of organic matter improved growth and yield of maize. Tryptophan addition with PGPR has augmented the seed number without effecting seed size. This was reflected in the enhanced growth and improved fertility status of the soil.

PGPR inoculation decreased the soil EC and tryptophan addition did not exert any significant effect on PGPR induced decline in soil EC. However, the tryptophan showed further decline on the PGPR mediated decrease in SAR. The results were more pronounced in saline sodic field than pots. The observed decrease in EC and SAR of rhizosphere soil of PGPR inoculated plants is attributed to the corresponding decrease in Na contents of the rhizosphere soil concomitant with an increase in K, Ca and Mg and organic matter contents. Bioinoculants used present study had the potential of decreasing the toxic ions (Na, Cl and HCO₃⁻) and reclaim the saline sodic soil by increasing P-solublization and increasing NO₃⁻–N availability. This effect was further augmented by tryptophan addition. The results obtained are in agreement with previous findings.

Under saline condition, excess Na competes with the K-uptake but still Na accumulation is higher in the rhizoplane of plants. Nutrient availability and uptake is to be enhanced by PGPR. It is reported that inoculating bacteria associated with the root zone, synthesize exoploysacchrides thereby preventing apoplastic flow of Na into the vascular tissue. Under salinity, the expression of ion transporters are changed by PGPR which ameliorate the toxic effects of Na and Cl either by changing soil pH or by improving nutrient availability. Previous results indicated that application of *Pseudomonas fluorescences* and *Enterobacter aerogenes* decrease Na contents in maize leaves under salt stress.
The increased phosphorus and NO$_3$-N contents in treated soil, with tryptophan and PGPR might be attributed to phosphate solubilization and N-fixing ability of *Pseudomonas moraviensis* and *Bacillus cereus*. Tryptophan addition to soil stimulated the ability of applied PGPR in improving N, P, K, and other nutrients. Etesami et al., also reported PGPR induced increase in N, P and K which was further augmented in presence of tryptophan. Auxin producing rhizobacteria improved soil fertility status by improving nutrient availability and organic carbon.

Addition of tryptophan with PGPR resulted in increase nutrients in soil and leaves. The magnitude of increase was higher under unstressed condition and potted plants. Under salt stressed condition the nutrient accumulation was higher in potted plants than in saline sodic field. The contribution of PGPR in improving plant growth in the presence or absence of tryptophan is directly correlated with the mechanism by which PGPR enhance the availability of nutrients (N, P and K).

Improvement of Ca and Mg in wheat leaves might be attributed to PGPR ability for balancing nutrients as resulted in inoculation of canola with *Artherobacter lipoferum*. The increase in nutrients uptake of PGPR treated plants is in agreement with previous findings. *Bacillus subtilis* and *Artherobacter* species improved P and K and decreased Na under salt stress in wheat.

The present study revealed the potential of *Pseudomonas moraviensis* and *Bacillus cereus* in the presence of tryptophan for improving plant height and fresh weight and these effects were more pronounced in unstressed condition. Similarly, the growth was better in field grown plants over potted plants both under stressed or unstressed conditions. The magnitude of stimulation in plant height was greater in *Bacillus cereus* treated with tryptophan. Tryptophan is the precursor for indole acetic acid, and the observed increase in plant height following
addition of tryptophan may be attributed to the PGPR induced conversion of tryptophan into indole acetic acid which may be attributed to IAA induced cell division and cell elongation.

The observed increased in fresh weight in the plants treated with Bacillus cereus + tryptophan, under saline sodic condition may be attributed to IAA induced water and nutrient uptake by plant. Increased fresh weight is indicative of better water/nutrient uptake which is as a strategy to mitigate/overcome the salt stress induced inhibition in roots. This may be due to efficient conversion of IAA which in turn assists in better proliferation of root system.

During the present study tryptophan addition increased the proline content in the leaves of treated plants. The magnitude of increase was greater in saline field grown plants over potted plants, grown under salt stress. Proline acts as a source of organic nitrogen reserve as well as osmoprotectant and antioxidant in such cases. PGPR have ability to increase proline and in such cases proline act as osmoprotectant, ROS scavenger as well as antioxidant.

The observed chlorophyll and proline contents may be due to the promoter role of PGPR in stomatal conductance, osmoregulation and photosynthesis. Proline also acts as a source of organic nitrogen reserve. Proline and IAA are useful markers for evaluating the bacterial efficiency under stressed conditions. There is strong correlation between IAA and proline production in axenic culture and previously Bacillus megaterium having these traits proved beneficial in the amelioration of dehydration stress.

Addition of tryptophan with PGPR augmented the activities of antioxidants by 50-100% and Bacillus cereus was more effective in this regard. During the present study tryptophan addition enhanced the antioxidant enzyme activities significantly and magnitude of increase was greater in field grown plants over potted plants, grown under salt stress. Antioxidant activities were increased by PGPR application because proline acts as ROS scavenger. Plant growth and development is modulated by the enzymes and phytohorhormones and PGPR
based mechanism is involved in direct synthesis of indole acetic acid, gibberelic acid and abscisic acid. The foliar application of tryptophan augmented the activities of antioxidant enzymes and PGPR inoculation further enhanced these activities. The magnitude of increase in antioxidants was higher in field grown plants because the interaction effects of climate with PGPR and plants produced more ROS. Hence, the tolerance strategy may be adapted to make antioxidant system more efficient to accumulate salt induce oxidative stress.

Tryptophan addition with *Pseudomonas moraviensis* and *Bacillus cereus* increased IAA contents by 2 fold in rhizosphere soil and 20% higher than control in leaves of plants grown with 150 mM NaCl. Increase in the level of IAA in the rhizosphere soil and leaves following inoculation with PGPR in presence of tryptophan demonstrate the PGPR induced modulation of IAA level in the inoculated plants and several genera of *Bacillus* and *Pseudomonas* are reported to be involved.

PGPR synthesizes IAA which was further augmented in presence of tryptophan. Foliar application of tryptophan was stimulatory to growth parameters of wheat under salt stress. IAA and ABA contents were higher in treated plants but ABA content (Table 3.24) of PGPR isolates as well as leaves of inoculated plants were 50% less than that of IAA. Increase in growth of wheat in the saline field could be attributed to enhanced physiological responses of crop with added PGPR isolates and tryptophan. Increase in the level of IAA in the rhizosphere soil and leaves following inoculation with *Pseudomonas moraviensis* and *Bacillus cereus* in presence and absence of tryptophan demonstrate the PGPR induced modulation of IAA level in the inoculated plants.

The PGPR inoculation modulated the IAA/ABA ratio in saline sodic field such that it was least (0.83) for rhizosphere soil of inoculated plants as compared to that in plant leaves (1.5).
Exposure of plants to salinity is known to induce a proportional increase in ABA concentration that is in most cases correlated with leaf or soil water potential.

Increase in yield attributes was higher in field grown plants and under unstressed condition. Similarly, the economic benefits were also greater for unstressed field grown wheat. Increase in spike length, grain yield and seed weight might be attributed to increase level of N, P and K in the presence of PGPR and tryptophan and increase rate of photosynthesis. The greater % increase in seed size of *Pseudomonas moraviensis* and *Bacillus cereus* inoculated plants may be attributed to higher IAA/ABA ratio in leaves which may account for increased biomass.

Addition of tryptophan stimulated the activities and efficiency of PGPR in the soil, by improving the ability to produce phytohormones. The observed higher increase in yield components might be attributed to the greater microbial and enzymatic activities and rapid release of the nutrients in the soil.

The application of tryptophan alone in the soil showed no significant changes on nutrients status, growth, physiology and yield parameters as compared to control but in combination with PGPR, these effects were many fold higher than control. *Bacillus cereus* addition with tryptophan was more beneficial than *Pseudomonas moraviensis*.

**3.4. Conclusions**

Addition of tryptophan at 0.21 mg/L to the culture media may boost up the proliferation of the PGPR as compared to control, measured as O.D at 660 nm. *Bacillus cereus* responded better to tryptophan addition in culture media and soil. *Bacillus cereus* also produced higher IAA in culture, salt stressed soil and leaves of treated plants. The higher IAA (growth promoting hormone) in the rhizosphere soil may assist the host plants interaction with the PGPR and subsequently induced tolerance against salt. The comparative effects of tryptophan alone and in the presence of PGPR revealed the potential of PGPR in ameliorating the
salinity. This was manifested by decreased in EC, Na/K, Na/Ca and SAR, of saline sodic field. IAA content was higher in culture media than soil. Tryptophan addition in the presence of PGPR often resulted in greater accumulation of IAA in culture or inoculated plants but little is known about changes in soil IAA. The accumulation of higher IAA and ABA, IAA/ABA ratio in soil and leaves of plants treated with tryptophan and grown under saline sodic field seems to be an adaptive mechanism of salt tolerance. The exploration of the other beneficial bacterial strains and their use in consortium and in the presence of tryptophan may prove beneficial for sustainable agriculture.

Chapter - 4

4. Introduction

Biofertilizers are defined as the formulations containing living microorganisms or latent cells having the potential of colonizing roots of crop plants and promote the growth by improving nutrients availability and acquisition.

Biofertilizer from nitrogen fixing bacteria come in three forms: liquid, solid and lyophilized. For liquid and lyophilized ones, only culture medium is used, but for solid form, carriers such as peat, activated charcoal and chicken dung are needed. Powder type biofertilizers are formulated by suspending carriers to liquids, and are directly applied to seeds or dipping of plants sprouting parts prior to sowing.

Improvement in soil fertility through fixing atmospheric Nitrogen, making the P available through P- solubilization and production of plant growth substances are the main outcome of a good biofertilizer. Long term use of biofertilizer is efficient, productive, and economical as well as eco-friendly.

Every year salt affected land is rigorously increasing adversely effecting agriculture and cropping system hence resulting in heavy economic losses. Salinity inhibits nutrient uptake of
plants due to accumulation of Na\(^+\), HCO\(_3\) and Cl\(^-\). Among several agricultural practices for salinity alleviation, application of biofertilizers has been proved promising for growth and yield of different crops.

Plant growth promoting rhizobacteria (PGPR) are basic components of biofertilizers and PGPR strains such as *Burkholderia*, *Enterobacter*, *Azospirillum*, *Azotobacter*, *Rhizobium*, *Erwinia* and *Flavobacterium* have proved very affective in this regard.

Some agronomically important *Pseudomonas* strains have been screened which have potential to improve seed germination, seed establishment and yield under normal and stressed environments. The commercial bioformulations comprising *Bacillus* are widely used in agriculture. Application of *Bacillus thuringiensis* in carrier based formulation has been reported.

The application of carrier based formulation of *Pseudomonas putida* enhanced growth and yield of wheat under salt stress. Similarly, the application of some PGPR was also very effective in improving plant growth under salt stress. Strains like *Bacillus subtillis* and *Pseudomonas corrugate* in addition with alginate beads, alginate beads + skim milk and charcoal showed increase in root and shoot length and dry weight of maize when applied in cooler region.

For preparation of stable biofertilizer suitable carrier (media used for multiplication of PGPR) is required because it act as carbon source and increase shelf life of biofertilizer. A good carrier material should be non-toxic sterilizeable, non-reactive, rich in organic matter and should have strong moisture holding capacity. Charcoal, lignite, Peat, farm yard manure (FYM), rice husk has been tested so far as carrier.

During last two decades, carrier based formulations remained a hot topic but due to certain constraints like the appliance of this industry is still not prominent. The lack of efficient, compatible microbial strains with synergistic behaviour for native microflora and insects
often hampered the formulation of effective product. Similarly the lack of awareness on farmers side, availability of suitable carriers, long term storage, transportation and commercialization have also halted the flourishing of biofertilizer industry.

The aim was to select efficient strain and the carrier to be used. The single stain interaction with carrier and their consortium to determine the synergistic effect of the strain were done. To determine the sole effect of PGPR the experiment was conducted under axenic condition in pots where salt stress was induced by applying 150 mM NaCl and saline sodic field condition. The effects of two PGPR isolates *Bacillus cereus* and *Pseudomonas moraviensis* were determined in different combinations with two carrier materials (maize straw and sugarcane husk) on soil health, physiology and yield of wheat. Similarly, the efficiency of these PGPR was also checked under axenic condition in pots and natural condition in field in normal unstressed soil.
4.1. Material and Methods

4.1.1. Soil preparation and sowing

Field Experiment

The soil in the field was ploughed and plots measuring 5 m$^2$ with row to row distance 20 cm were prepared. No chemical fertilizer was added but adequate NO$_3$-N, P and K were available in soil (Table 4.1). For each treatment four replicates were used in Randomized Complete Block Design (RCBD). Seeds coated with biofertilizer were sown by hand drill method.

Pot Experiment

Earthen pots measuring 17x20 cm$^2$ were filled with autoclaved soil (garden soil) and sand mixed in 3:1 ratio. Salinity was induced by adding 150 mM NaCl (EC= 3.6 dS m$^{-1}$) with irrigated water after 7d and 14d of sowing. Following treatments were made both for field and pot experiment.

4.1.2. Method of biofertilizer preparation and application

4.1.3. Preparation of inocula

Two isolates Pseudomonas moraviensis (accession No. LN714047) and Bacillus cereus (accession No. LN714048) from rhizospheric soil of halophytic herbs Chrysopogon aucheri and Cenchrus ciliaris were applied on wheat for two consecutive years. Liquid culture of Pseudomonas moraviensis and Bacillus cereus were prepared by growing PGPR on L.B media for 7d (10$^8$ cfu/mL and O.D ~ 1 at 600 nm). The coinoculation of two PGPR was made on the basis of synergistic behaviour of the strains tested in the culture.

4.1.4. Formulation of biofertilizer
Maize straw and sugarcane husk used as carriers were shade dried at room temperature (22-25 °C) and sieved through 0.20-0.31 mm sieve (ANTAI China). Carriers were sterilized by autoclave. The carrier (50 g) previously sterilized by autoclaving was inoculated with 20 ml of liquid broth having (8.5–9.9 × 10^9 cells ml^−1) with 1% molasses. Product thus formed was incubated for 24 h in laminar flow, packed and sealed.

The formulations contained 13x10^8 cfu g^−1 and 19x10^8 cfu g^−1 carrier for *Pseudomonas moraviensis* and *Bacillus cereus* respectively measured after 3 weeks of incubation (Fig 1).

4.1.5. Inoculation studies

The seeds of wheat (Inqlab-91) were surface sterilized with 70% ethanol for 2 min followed by shaking with 10% chlorox for 2 - 3 min. The seeds were successively washed 3-4 times with autoclaved distilled water. The biofertilizer (2 g) was mixed with 100 ml autoclaved water which was sufficient for 250 g seeds. Seeds were soaked for 1 h and shade dried for 15 min prior to sowing. The average temperature of the growing area was 25 ± 2ºC with 11 h photo period and 13 h dark period; humidity varied from 75 - 80%.

4.1.5. P-solublization potential of PGPR

Pure colonies of bacterial strains were pin point inoculated on sterilized Pikovskaya agar. Appearance of halo zones around some of the colonies indicated phosphate solubilizing ability. Bacterial colonies were isolated and were inoculated separately into conical flasks containing Pikovskayas broth and incubated at room temperature (25 ± 2ºC) on an orbital shaker for 6 d. The cultures were centrifuged at 8000 g for 20 min at room temperature (25 ± 2ºC) and 2 ml aliquots of the supernatant were used to detect soluble phosphorous using chloromolybdic acid - stannous chloride method at 882 nm. The corresponding amount of soluble phosphate was calculated from a standard curve of KH₂PO₄.

5.1.6. Antifungal activity of PGPR
The agar tube dilution method was used for the determination of antifungal activity of extract. Percentage inhibition of fungal growth for each PGPR isolates was determined against *Helminthosporium sativum* and *Fusarium moniliforme* as;

\[
\text{Percentage inhibition of fungal growth} = 100 - \frac{\text{Linear growth in test sample (mm)} \times 100}{\text{Linear growth in control (mm)}}.
\]

### 4.2. Results

#### 4.2.1. Colony forming unit of PGPR in carrier

Colony forming Unit (cfu) of both *Pseudomonas moraviensis* and *Bacillus cereus* (Fig 4.1) increased linearly with incubation period irrespective of the carrier material used in the biofertilizer. *Bacillus cereus* showed 25% higher cfu in sugarcane husk at 7d, 21d and 40d of inoculation. The coinoculation of *Pseudomonas moraviensis* with *Bacillus cereus* (Fig 5.2) resulted in 60% increase in cfu as compared to single inoculation made 7d after inoculation.

#### 4.2.2. Colony forming unit of PGPR in unstressed soil

Both *P. moraviensis* and *B. cereus* were able to survive in sterilized soil after 57d of sowing (Fig 4.3). *P. moraviensis* showed better survival in maize straw and sugar husk as compared to *B. cereus* when inoculated alone (PS+MZ, PS+SC, BC+MZ, BC+SC).

Single inoculation of *P. moraviensis* in sugarcane husk and maize straw exhibited 23% and 42% less cfu as compared to coinoculation in these carriers. Percentage increase was higher in sugarcane husk. *B. cereus* and *P. moraviensis* coinoculation in sugarcane husk and maize straw also showed 27% and 48% greater cfu respectively over that of single inoculation in the same carrier. In maize straw the cfu g\(^{-1}\) of both the PGPR were lower than that of sugarcane husk based biofertilizer and this was also evident in coinoculation.

#### 4.2.3 Colony forming unit of PGPR in stressed soil

*Pseudomonas moraviensis* and *Bacillus cereus* survived after 57d of inoculation in rhizosphere soil of pots grown plants grown under salt stress (Fig 5.4). *Bacillus cereus*
showed 18% and 24% better survival efficiency measured as colony forming unit (cfu) in maize straw and sugarcane husk respectively over *Pseudomonas moraviensis*.

### 4.2.4. Antifungal activity of PGPR strains

Both *Pseudomonas moraviensis* and *Bacillus cereus* inhibited the growth of two pathogenic fungi *Fusarium moniliforme* and *Helminthosporium sativum* (Fig 4.5). Both the PGPR showed (58%) inhibition against *Helminthosporium sativum*. *Bacillus cereus* was equally effective against both the fungal pathogens with 52% inhibition.

### 4.2.5. P- solublization of PGPR

Both the strains were phosphate solublizer but *Pseudomonas moraviensis* had 14% greater potential than *Bacillus cereus* (Table 4.4).

### 4.2.5. Effects of biofertilizer on physicochemical characteristics and nutrient status of rhizosphere soil of wheat grown under salt stressed conditions

Data of 2010-2011 had been described whereas the data of 2011-2012 have also been presented for comparison. The electrical conductivity (EC) of the rhizosphere soil of potted plants treated with 150 mM NaCl was decreased by 30% (Tables 4.5 and 4.6), when consortium of PGPR was applied with carriers (PS+BC+MZ and PS+BC+SC).

During 2010-2011 under saline sodic field condition, *Pseudomonas moraviensis* and *Bacillus cereus* consortium with carriers decreased the electrical conductivity (EC) of the saline sodic soil by 21% over control.

Organic matter of soil of potted plants was increased by 30% in coinoculation (PS + BC) and application of PGPR consortium with carriers (PS+BC+SC) (Table 4.5). In saline sodic field, single inoculation of PGPR with carriers (PS+MZ, PS+SC, BC+MZ, BC+SC) increased
organic matter by 28% over control. Coinoculation of PGPR and addition of carrier with PGPR further increased organic matter by 12%.

The decrease in sodium absorption ratio (SAR) of rhizosphere soil (Table 4.5) was 22% lower over control when consortium of two PGPR were applied without carrier (PS + BC) and further 13% decrease occurred when consortium was mixed with sugarcane husk (PS+BC+SC) and maize straw (PS+BC+MZ). Similar trend was followed during 2011-2012 (Table 4.6).

In pots grown plants decrease in Na contents of rhizosphere soil was 30% over control in biofertilizer treatment (Table 4.5). The coinoculation of PGPR (PS + BC) exhibited 10% less Na, Cl and HCO₃⁻ in the rhizosphere soil (Tables 4.8 and 4.9) and % decrease was even greater (13-19 %) when both PGPR were applied with carriers (maize straw and sugarcane husk) in the form of biofertilizer.

Phosphorus, NO₃⁻-N and potassium contents of rhizospheric soil (Tables 4.5 and 4.6) were higher in all the treatments of pots grown plants. Single Inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with carriers increased 30-40% NO₃⁻-N, P and K contents over control which was further increased by 6-14% when two PGPR were coinoculated with carriers. *Bacillus cereus* was more efficient while sugarcane husk was better carrier for improving NO₃⁻-N, P and K contents of soil. The Ca and Mg contents were significantly higher only when *Pseudomonas moraviensis* and *Bacillus cereus* were applied in coinoculation with carriers.

Single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with carriers augmented, P and NO₃⁻-N contents of rhizosphere soil of field (Table 4.8) by 28% and 35% over control. The PGPR consortium with carrier showed further 10% and 20% increases in NO₃⁻-N and P contents respectively. NO₃⁻-N contents were 30% and 44% higher in field grown plants under
stressed condition (Table 4.8) over potted plants (Table 4.5), following the coinoculation of PGPR and application of PGPR with carriers.

Under stressed condition potted plants exhibited 25% increase in P content over field grown plants grown under saline sodic condition (Table 4.8), following the coinoculation and application of PGPR consortium with carriers.

The K and Mg contents (Table 4.8) of saline sodic soil were improved by 28% and 41% in single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with maize straw and sugarcane husk. Consortium of PGPR further augmented K, Ca and Mg by 35%, 19% and 47%. Biofertilzer treatment improved K, Ca and Mg contents by 57%. Similar trend was followed during 2011-2012. K contents were 22% and 39% higher in potted plants under stressed condition (Table 4.5) over plants grown under saline sodic field (Table 4.8), following the coinoculation of PGPR and application of PGPR with carriers respectively.

The potted plants grown under stressed condition (Table 4.5), receiving single inoculation of PGPR with carriers had 35% higher Ca in rhizosphere soil over saline sodic field grown plants (Table 4.8). Coinoculation of PGPR increased 44% Ca content over control in potted plants treated with NaCl. The % increase over control in Ca content was 32% in stressed potted plants over plants grown under saline sodic field, following the treatment of PGPR consortium with carriers.

The potted plants grown under stressed condition (Table 4.5), receiving coinoculation of PGPR had 5% higher Mg in rhizosphere soil over saline sodic field grown plants (Table 4.8). Coinoculation of PGPR increased 40% Mg content over control both in potted and field grown stressed plants. The % increase over control in Mg content was 37% in stressed potted plants over plants grown under saline sodic field, following the treatment of PGPR consortium with carriers. Similar trend was followed during 2011-2012.
The $\text{Na}^+/\text{K}^+$ and $\text{Na}^+/\text{Ca}^+$ ratio were 25-30% less over control when PGPR were used in consortium and further 10-15% decrease was discerned in biofertilizer treatment. Similar trend was followed during 2011-2012.

4.2.6. Effects of biofertilizer on physicochemical characteristics and nutrient status of rhizosphere soil of wheat grown under unstressed conditions

During 2010-2011 increase in soil organic matter was significantly higher (58% and 29%) over control in rhizosphere of pots and field grown plants respectively (Table 4.10 and Table 4.11), when *Pseudomonas moraviensis* was inoculated with maize straw and sugarcane husk. Addition of *Bacillus cereus* with carriers increased (82% and 40%) organic matter in pots and field grown plants respectively. The % increase in organic matter when both PGPR were used in consortium was 96% higher in pots grown plants and 50% in field grown plants. Coinoculation of two PGPR with maize straw and sugarcane husk (biofertilizer treatment) further increased 75% and 30% organic matter in pots and field grown plants over PGPR consortium (without carrier). In field grown unstressed plants (Table 4.11), single inoculation of PGPR increased organic matter by 33% over stressed plants. Coinoculation of PGPR and application of PGPR consortium with carriers increased organic matter by 62% and 131% respectively.

In potted plants grown under unstressed condition (Table 4.10), single inoculation of PGPR increased organic matter by 40% over potted plants grown under salt stress (Table 4.5). Coinoculation of PGPR increased 80% organic matter under unstressed potted plants. The increase in organic matter was 140% in unstressed potted plants over stressed potted plants when PGPR consortium was applied with carriers. Similar trend was followed during 2011-2012.
During 2010-2011 in potted and field grown plants under unstressed condition (Table 4.10 and Table 4.11), NO$_3$-N contents of soil were 55-60% and 25% higher over control when *Pseudomonas moraviensis* and *Bacillus cereus* were singly inoculated with maize straw and sugarcane husk.

Increase in NO$_3$-N was 33% higher under unstressed condition in single inoculation, coinoculation and application of PGPR consortium with carriers (Table 4.11). Similarly in field grown unstressed plants single inoculation of PGPR increased P by 32% over stressed field plants (Table 4.8). Coinoculation of PGPR and application of PGPR consortium with carriers increased NO$_3$-N by 10% and 21% respectively.

The potted plants grown under unstressed condition (Table 4.10) receiving single inoculation of PGPR with carriers had 10% higher NO$_3$-N in rhizosphere soil over potted plants grown under salt stress (Table 4.5). Coinoculation of PGPR increased 8% NO$_3$-N over control under unstressed potted plants. The % increase over control in NO$_3$-N was 5% in unstressed potted plants over stressed potted plants following the treatment of PGPR consortium with carriers.

Similar trend was followed during 2011-2012.

During 2010-2011 plants grown in pots (Table 4.10) and field (Table 4.11) under unstressed condition, single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with maize straw and sugarcane husk increased P contents by 30-40% in pots grown plants and 50% in field grown plants. Coinoculation of both PGPR increased P contents by 45% both in pots and field grown plants. Biofertilizer treatment increased 77% and 65% P in pots and field grown plants respectively. P content was increased by 30% over control in single inoculation in potted plants grown under unstressed condition (Table 4.10) over potted plants grown under stressed condition (Table 4.5). Coinoculation of PGPR increased P content by 24% in
unstressed potted plants. PGPR consortium with carriers equally increased (74%) P contents over control under stressed and unstressed condition.

The K contents were 40% higher in pots grown plants (Table 4.10) and 25% higher in field grown plants (Table 4.11) when *Pseudomonas moraviensis* and *Bacillus cereus* were singly inoculated with maize straw and sugarcane husk. In coinoculation, increase in K content was 56% higher in pots grown plants and 29% in field grown plants. Application of biofertilizer further increased K content by 22% and 7% in pots and field grown plants respectively. K content was increased by 12% over control in single inoculation in field grown plants under unstressed condition (Table 4.11) over field grown plants under stressed condition (Table 4.8). Coinoculation of PGPR increased K content by 10% and this increase was 43% higher when PGPR consortium was applied with carriers.

The Ca content were 30-40% higher in the rhizosphere soil of both field and pots grown plants (Table 4.10 and Table 4.11) when maize straw and sugarcane husk were added with *Pseudomonas moraviensis* and *Bacillus cereus*. PGPR consortium increased Ca contents by 64% and 42% in pots and field grown plants and further 18% increase was recorded both in pots and field grown plants when bifertilizer was applied (carrier based consortium).

Ca content was increased by 7% over control in single inoculation in potted plants grown under unstressed condition (Table 4.10) over potted plants grown under stressed condition (Table 4.5). Coinoculation of PGPR increased Ca content by 8% in unstressed potted plants. PGPR consortium with carriers equally increased (80%) Ca contents over control under stressed and unstressed condition.

*Bacillus cereus* inoculation with maize straw and sugarcane husk increased Mg contents of soil by 45% in rhizosphere soil of both pots and field grown plants (Tables 4.10 and Table 4.11). *Pseudomonas moraviensis* showed 60% higher Mg contents in pots grown plants and
47% in field grown plants. Consortium of two PGPR increased Mg contents by 86% and 61% in pots and field grown plants respectively. Biofertilizer application further increased (15% and 30%) Mg contents in potted and field grown plants respectively. Similar trend was followed during 2011-2012.

Similarly in field grown under unstressed condition, single inoculation of PGPR increased Mg by 15% over stressed field plants (Table 4.8). Coinoculation of PGPR and application of PGPR consortium with carriers increased Mg by 47% and 58% respectively. Single inoculation with carriers, coinoculation and application of PGPR with carriers increased Mg content by 20% in potted plants grown under unstressed condition (Table 4.10) over potted plants grown under stressed condition (Table 4.5).

### 4.2.7. Effects of biofertilizer on nutrient accumulation in wheat leaves under salt stress

The single inoculation of both the PGPR (*Pseudomonas moraviensis* and *Bacillus cereus*) in potted plants increased NO$_3$-N, P, Ca and Mg accumulation of treated leaves (Table 4.12). Phosphorus, NO$_3$-N and calcium contents of leaves were 35-47% higher in single inoculation. Coinoculation of both the PGPR and addition of carrier materials showed no further increase in uptake of these nutrients. Coinoculation of both the PGPR and their combination with maize straw and sugarcane husk significantly (20-24%) increased Ca in soil. Similar trend was followed during 2011-2012.

The formulation of biofertilizer in the presence and absence of carrier was effective in decreasing Na accumulation in pots grown plants under salt stress during 2010-2011 (Table 4.11). Single inoculation of PGPR with carrier materials significantly decreased (28%) Na contents of leaves. Coinoculation of two PGPR with both the carriers further decreased (15-17%) Na accumulation. Na contents of leaves were 15% higher in potted plants under stressed condition (Table 4.12) over plants grown under saline sodic field (Table 4.13).
following the single inoculation of PGPR with carriers and coinoculation of PGPR respectively.

During 2010-2011 under saline sodic field condition, application of *Pseudomonas moraviensis* and *Bacillus cereus* increased K uptake of leaves by 25% (Tables 4.13 and 4.14) when mixed separately with maize straw and sugarcane husk. Increase in Ca was 22% when sugarcane husk was used as carrier with two PGPR and this increase was 40% when maize straw was mixed with the PGPR. PGPR consortium improved K and Ca accumulation in leaves by 34% and 57% over control. Biofertilizer treatment improved K, Ca and Mg contents of leaves by (30-45%) over control and inhibited the Na accumulation by 36% over control.

The potted plants grown under stressed condition, receiving single inoculation of PGPR with carriers had 14% higher K in leaves over saline sodic field grown plants (Table 4.13). The potted plants (Table 4.12) exhibited 6% increase in k content over field grown plants under saline sodic condition, following the coinoculation and application of PGPR consortium with carriers.

Under stressed condition, single inoculation of PGPR with carriers equally increased the Ca content of leaves over control in potted and field grown plants (Table 4.12 and Table 4.13). The % increase over control in Ca content was 14% in stressed potted plants over plants grown under saline sodic field, following the coinoculation and application of PGPR consortium with carriers.

The potted plants grown under stressed condition (Table 4.12) receiving single inoculation of PGPR with carriers increased 10% Mg in leaves over saline sodic field grown plants (Table 4.13 and Table 4.14). The potted plants exhibited 24% and 28% increase in Mg content over
field grown plants under saline sodic condition, following the coinoculation and application of PGPR consortium with carriers respectively.

The Na/K and Na/Ca of leaves (Table 4.13 and 4.14) were decreased by 26-45% and 41-55% when PGPR were inoculated separately with carriers. Coinoculation of PGPR decreased Na/K by 69% and Na/Ca by 98%. Biofertilizer treatment further decreased the Na/K and Na/Ca by 25% and 61% respectively. Similar trend was followed during 2011-2012.

4.2.8. Effects of biofertilizer on nutrient accumulation in wheat leaves under unstressed condition

During 2010-2011 under unstressed condition, *Pseudomonas moraviensis* addition with maize straw and sugarcane husk increased, NO₃-N contents of leaves by 90% over control in pots grown plants (Table 4.15) and 50% in field grown plants (Table 4.16). This increase was 103% in pots and 57% in field grown plants when *Pseudomonas moraviensis* was used singly. Consortium of PGPR increased 115% NO₃-N over control both in pots and field grown plants. Biofertilizer treatment showed 64% and 25% further increase in NO₃-N in pots and field grown plants respectively.

The potted plants grown under unstressed condition (Table 4.15) receiving single inoculation of PGPR with carriers accumulated 39% higher NO₃-N in leaves over potted plants grown under salt stress (Table 4.13). Coinoculation of PGPR increased 27% NO₃-N over control under unstressed potted plants. The % increase over control in NO₃-N was 80% in unstressed potted plants over stressed potted plants following the treatment of PGPR consortium with carriers.

Phosphorus contents of leaves were increased by 80-85% in pots grown plants (Table 4.15) and 40% in field grown plants under unstressed condition (Table 4.17), when *Pseudomonas*
*Pseudomonas moraviensis* and *Bacillus cereus* were added with sugarcane husk and maize straw. Coinoculation of both PGPR showed significant increase (67%) in P contents in field grown plants. Biofertilizer application exhibited 115% higher P in pots grown plants and 85% in field grown plants. In potted plants grown under unstressed condition or stressed condition (Table 4.15 and Table 4.17), single inoculation of PGPR increased P content by 80% over control. Coinoculation of PGPR and application of PGPR consortium with carriers under unstressed condition increased P content by 21% over stressed potted plants.

Potassium (K) contents of leaves were 52% higher in pots (Table 4.15) and 40% in field grown plants (Table 4.17), when *Pseudomonas moraviensis* was used as bioinoculant with maize straw and sugarcane husk. This increase was significantly higher (88%) when *Bacillus cereus* was inoculated with the carriers. The increase in K content was 99% and 53% over control when consortium of two PGPR was applied in pots and field grown plants respectively. Biofertilizer treatment further enhanced K uptake by 18-25% in pots and field grown plants respectively. In field grown under unstressed condition (Table 4.17), single inoculation of PGPR increased K content by 4% over plants grown under saline sodic field (Table 4.14 and Table 4.15). Coinoculation of PGPR was equally effected with 50% increase over control in stressed and unstressed field grown plants. PGPR consortium with carriers increased K by 15% in unstressed field grown plants.

The potted plants grown under unstressed condition receiving single inoculation of PGPR with carriers had 36% higher K in leaves over potted plants grown under salt stress (Table 4.13). Coinoculation of PGPR increased 72% K over control in unstressed potted plants. The % increase over control in K content was 68% in unstressed potted plants (Table 4.15) over stressed potted plants (Table 4.13) following the treatment of PGPR consortium with carriers.
Increase in Ca contents was significantly higher (40%) over control in pots grown plants (Table 4.15) when *Pseudomonas moraviensis* and *Bacillus cereus* were inoculated with maize straw and sugarcane husk. The % increase in Ca contents was 50% higher when consortium of two PGPR was used in pots and field grown plants (Table 4.17). Biofertilizer treatment further increased Ca contents by 15-23% in pots and field grown plants.

In field grown under unstressed condition (Table 4.17), single inoculation of PGPR increased Ca content by 6% over plants grown under saline sodic field (Table 4.14 and Table 4.15). Coinoculation of PGPR increased 10% Ca in unstressed field grown plants. PGPR consortium with carriers increased Ca by 7% in unstressed field grown plants.

The Ca content of leaves was increased by 24% over control in single inoculation in potted plants grown under unstressed condition (Table 4.16) over potted plants grown under stressed condition (Table 4.17). Coinoculation of PGPR increased Ca content by 9% in unstressed potted plants. PGPR consortium with carriers equally increased (78%) Ca contents over control under stressed and unstressed condition.

During 2010-2011 under unstressed condition, potted and field grown plants (Table 4.15 and Table 4.16) accumulated 45% higher Mg in single inoculation of PGPR with maize straw and sugarcane husk. Coinoculation of two PGPR increased 56% and 69% Mg in pots and field grown plants respectively. The highest increase (90%) was observed both in pots and field grown plants in biofertilizer treatment. In field grown under unstressed condition (Table 4.15), single inoculation of PGPR increased Mg content by 27% over plants grown under saline sodic field (Table 4.14 and Table 4.15). Coinoculation of PGPR increased 48% Mg in unstressed field grown plants. PGPR consortium with carriers increased Mg by 70% in unstressed field grown plants. Similar trend was followed during 2011-2012.
The potted plants grown under unstressed condition (Table 4.15) receiving single inoculation of PGPR with carriers had 33% higher Mg in leaves over potted plants grown under salt stress (Table 4.13). Coinoculation of PGPR increased 11% Mg over control in unstressed potted plants. The % increase over control in Mg content was 30% in unstressed potted plants over stressed potted plants following the treatment of PGPR consortium with carriers.

4.2.9. Effects of Biofertilizer on wheat growth under salt stress

Increase in plant height (Table 4.17) was greater over control in potted plants grown under salt stressed condition in all the inoculation treatments using both types of carriers (sugarcane and maize straws). *Pseudomonas moraviensis* and *Bacillus cereus* addition as PGPR with both carrier increased the plant height by 42%. Coinoculation of PGPR increased the plant height by 54% over control. The magnitude of increase in plant height was higher when PGPR consortium was applied sugarcane husk was used as carrier either in single or coinoculation. Similar trend was followed during 2011-2012 (Table 4.18).

During 2010-2011 under saline sodic field condition, single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with carriers increased 20-25% plant height (Table 4.19). The increase was higher (39%) over control when PGPR were coinoculated and the biofertilizer comprising PGPR consortium and both carriers further stimulated fresh weight by 14%.

The single inoculation of PGPR with carriers and coinoculation of PGPR under saline sodic field (Table 4.19) increased 15% plant height over potted plants grown under salt stress (Table 4.17). Increase in plant height of the field grown plants was 19% higher over potted plants, following the coinoculation of PGPR and application of PGPR with carriers. Similar trend was followed during 2011-2012 (Table 4.18 and Table 4.20).
During 2010-2011, significantly higher fresh weight (20%) (Table 4.17) was observed over control when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated with both types of carriers.

During 2010-2011 under saline sodic field condition, significantly higher fresh weight (Table 4.19) was observed over control when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated singly with maize straw and sugarcane. Consortium of two PGPR increased fresh weight of aerial parts by 60%. Maximum increase (70-80%) was observed in biofertilizer treatment.

In field grown under stressed condition (Table 4.19), % increase in fresh weight was 33% higher over field grown plants under unstressed condition (Table 4.25). Coinoculation of PGPR increased 33% fresh weight in field grown plants under saline sodic condition. PGPR consortium with carriers increased fresh weight by 21% in saline sodic field over unstressed field grown plants.

The saline sodic field grown plants (Table 4.19) receiving single inoculation of PGPR exhibited 44% higher fresh weight over potted plants (Table 4.17). Coinoculation of PGPR increased 70% fresh weight over control in field grown stressed plants. The % increase over control in fresh weight was 55% in plants grown under saline sodic field over potted plants grown under salt stress, following the treatment of PGPR consortium with carriers. Similar trend was followed during 2011-2012 (Table 4.18 and Table 4.20).

**4.2.10. Effects of biofertilizer on chlorophyll content of leaves under salt stress**

During 2010-2011, the chlorophyll contents (Table 4.17) were 10-13% higher in potted plants grown under salt stress. Maximum increase (48%) was observed over control, when the consortiums of the two PGPR species were mixed with maize straw and sugarcane husk. Similar trend was followed during 2011-2012 (Table 4.18).
During 2010-2011, under saline sodic field condition, single inoculation of PGPR with sugarcane husk and maize straw increased chlorophyll content (Table 4.17) by 30% over control. The coinoculation of both PGPR showed 45% increase in chlorophyll contents of leaves. Significantly higher (48%) chlorophyll contents were observed in biofertilizer treatment both in pots and field grown plants.

Single inoculation with carriers, coinoculation and application of PGPR with carriers increased chlorophyll content by 24% over potted plants grown under stressed condition (Table 4.17 and Table 4.18). In field grown under stressed condition (Table 4.19), % increase in chlorophyll was 18% higher over unstressed field grown plants (Table 4.25). PGPR consortium with carriers increased chlorophyll content by 21% in saline sodic field over unstressed field grown plants.

The saline sodic field grown plants (Table 4.19) receiving single inoculation of PGPR exhibited 20% higher chlorophyll contents over potted plants (Table 4.17). Coinoculation of PGPR increased 40% chlorophyll over control in field grown stressed plants. The % increase over control in chlorophyll content was 35% in plants grown under saline sodic field over potted plants grown under salt stress, following the treatment of PGPR consortium with carriers.

### 4.2.12. Effects of biofertilizer on protein contents of leaves under salt stress

During 2010-2011 protein content of leaves (Table 4.17) were higher both in single and coinoculation. % increase was higher when *Bacillus cereus* was applied as single and in coinoculation treatment with both types of carriers. The consortium of the two PGPR (*Pseudomonas moraviensis* and *Bacillus cereus*) with carriers increased protein content of leaves by 45-50%. Similar trend was followed during 2011-2012 (Table 4.18).
The % increase over control in protein content of potted plants grown under stressed condition (Table 4.17) was 4% and 20% higher over potted plants grown under unstressed condition (Table 4.23), when PGPR were coinoculated and consortium of PGPR was applied with carriers respectively.

The single inoculation of PGPR with carriers and coinoculation of PGPR under saline sodic field condition (Table 4.19) increased 6% and 14% protein content over potted plants grown under salt stress (Table 4.17). Increase in protein content of the field grown plants was 10% higher over potted plants, following the application of PGPR with carriers.

4.2.13. Effects of biofertilizer on proline contents of leaves under salt stress

During 2010-2011, proline contents (Table 4.17) were significantly higher over control in all the treatments. There was no significant difference between the two PGPR in proline production when they were singly inoculated with carriers. The consortium of *Pseudomonas moraviensis* and *Bacillus cereus* showed 10-20% higher proline contents over that of each of the single strain applied. The consortium of *Pseudomonas moraviensis* and *Bacillus cereus* increased proline contents by 34% and 40%. The extent of increase was not dependent on the type of carrier. Similar trend was followed during 2011-2012 (Table 4.18).

During 2010-2011 under saline sodic field condition, proline contents of leaves (Table 4.19) were 22-27% higher when two PGPR were singly inoculated with carriers. Coinoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased proline contents by 38%. The addition of carrier material had no further effect on the proline production as compared to coinoculation. Similar trend was followed during 2011-2012 (Table 4.20).

In field grown under stressed condition (Table 4.19), % increase in proline content was 100% higher over field grown plants grown under unstressed condition (Table 4.25) Coinoculation
of PGPR increased 92% proline in field grown plants under saline sodic condition. PGPR consortium with carriers increased proline by 90% in saline sodic field over unstressed field grown plants. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.26).

In potted plants grown under stressed condition (Table 4.17), single inoculation of PGPR increased proline content by 5% over potted plants grown under unstressed condition (Table 4.23). Coinoculation of PGPR increased 16% proline in potted plants grown under stressed condition. The increase in proline content was 32% in stressed potted plants over unstressed potted plants when PGPR consortium was applied with carriers.

The saline sodic field grown plants (Table 4.19) receiving single inoculation of PGPR exhibited 39% higher proline contents over potted plants (Table 4.17). Coinoculation of PGPR increased 14% proline over control in field grown stressed plants. The observed increase over control in proline content was 10% in plants grown under saline sodic field over potted plants grown under salt stress, following the treatment of PGPR consortium with carriers. Similar trend was followed during 2011-2012 (Table 4.18 and Table 4.20).

4.2.14. Effects of biofertilizer on antioxidant enzymes activities of leaves under salt stress

During 2010-2011 plants grown in pots under salt stress, SOD and POD (Table 4.17) activities of plants treated with biofertilizer. In single inoculation, increase in SOD was higher when *Bacillus cereus* was added with sugarcane husk and POD activity was higher when *Pseudomonas moraviensis* was added with maize straw. Plants treated with biofertilizer comprising *Bacillus cereus* and *Pseudomonas moraviensis* with both carriers exhibited 57% and 45% higher SOD and POD activities over control. Similar trend was followed during 2011-2012 (Table 4.18).
During 2010-2011 under saline sodic field condition, superoxide dismutase (SOD) and peroxidase (POD) (Table 4.19) activities of leaves were increased by two fold in single as well as in coinoculation of Bacillus cereus and Pseudomonas moraviensis. Increased in POD was 126% higher over control when Pseudomonas moraviensis and Bacillus cereus were coinoculated with or without carriers. Similar trend was followed during 2011-2012 (Table 4.20).

In field grown under stressed condition (Table 4.19), % increase in SOD activity was 109% higher over field grown plants grown under unstressed field (Table 4.25). Coinoculation of PGPR increased 91% SOD activity in field grown plants under saline sodic condition. PGPR consortium with carriers increased SOD activity by 116% in saline sodic field over unstressed field grown plants. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.26).

In potted plants grown under stressed condition (Table 4.17), single inoculation of PGPR increased SOD activity by 44% over potted plants grown under unstressed condition (Table 4.23). Coinoculation of PGPR increased 55% SOD in potted plants grown under stressed condition. The increase in SOD activity was 97% in stressed potted plants over unstressed potted plants when PGPR consortium was applied with carriers. Similar trend was followed during 2011-2012 (Table 4.18 and Table 4.24).

The SOD activity of saline sodic field grown plants (Table 4.19) was 15% higher over potted plants (Table 4.17) receiving single inoculation of Bacillus cereus and Pseudomonas moraviensis. Coinoculation of PGPR was also more effective under saline sodic field with 6% higher SOD activity over potted plants. The application of PGPR consortium with carriers increase SOD activity by 140% both in potted and field grown plants grown under
stressed condition. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.18).

In field grown under salt stressed condition (Table 4.19), % increase in POD activity was 111% higher over field grown plants grown under unstressed field (Table 4.25). Coinoculation of PGPR increased 124% POD activity in field grown plants under saline sodic condition. PGPR consortium with carriers increased POD activity by 139% in saline sodic field over unstressed field grown plants. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.26).

In potted plants grown under stressed condition (Table 4.17), single inoculation of PGPR increased POD activity by 49% over potted plants grown under unstressed condition (Table 4.23). The increase in POD activity was 60% in stressed potted plants over unstressed potted plants when PGPR were coinoculated or consortium of PGPR was applied with carriers. Similar trend was followed during 2011-2012 (Table 4.18 and Table 4.24).

The POD activity of saline sodic field grown plants (Table 4.19) was 8% higher over potted plants (Table 4.17) receiving single inoculation of *Bacillus cereus* and *Pseudomonas moraviensis*. Coinoculation of PGPR was also more effective under saline sodic field with 40% higher POD activity over potted plants. The application of PGPR consortium with carriers increased POD activity by 24% both in potted and field grown plants grown under stressed condition. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.18).

**4.2.15. Effects of biofertilizer on indoleacetic acid (IAA), gibberellic acid (GA) and abscisic acid (ABA) contents of leaves under salt stress**
During 2010-2011 plants grown in pots under salt stress, indole acetic acid (IAA), gibberellic acid (GA) and abscisic acid (ABA) contents of leaves were 35-50% higher (Table 4.21) in single inoculation of *Pseudomonas moraviensis* and *Bacillus cereus* with both type of carriers. Coinoculation of both PGPR also improved the phytohormone content of leaves. The consortium of 2 PGPR with maize straws and sugarcane husk further improved (6-8%) IAA and GA contents. Similarly, the ABA content was enhanced by 15% over single inoculation when PGPR were applied with carriers. Similar trend was followed during 2011-2012 (Table 4.21).

During 2010-2011 under saline sodic field condition, indole acetic acid (IAA) and gibberellic acid (GA) contents (Table 4.21) were 61% and 88% higher when two PGPR were inoculated with carries separately. Coinoculation of *Pseudomonas moraviensis* and *Bacillus cereus* increased IAA and GA contents by 48% and 76% respectively irrespective of the carrier material. Similar trend was followed during 2011-2012.

During 2010-2011 under saline sodic field condition, single inoculation of PGPR increased abscisic acid (ABA) significantly (85-92%) over control (Table 4.21). Coinoculation of both PGPR without carrier showed 129% higher ABA over control. Addition of carrier material to the bioinoculants i.e in the form of biofertilizer further enhanced ABA contents by 6-7% than that of without carrier. Similar trend was followed during 2011-2012.
4.2.16. Effects of Biofertilizer on wheat growth under unstressed condition

During 2010-2011 plants grown in pots and field under unstressed condition (Table 4.22 and Table 4.24), PGPR Inoculation with maize straw and sugarcane husk increased plant height in all treatments both in pots and field grown plants. Single inoculation of *Bacillus cereus* and *Pseudomonas moraviensis* with maize straw and sugarcane husk increased plant height by 28-33% in pots and 23-34% in field grown plants. As carrier sugarcane husk with both PGPR showed better results. Coinoculation of both PGPR increased plant height significantly by 53% and 39% in pots and field grown plants respectively. Maximum increase 58% in pots and 48% in field were observed in biofertilizer treatment.

In potted plants grown under stressed and unstressed condition (Table 4.17, Table 4.22), single inoculation of PGPR increased plant height equally by 40% over control. Coinoculation of PGPR increased 3% plant height in unstressed potted plants. The increase in plant height was 12% in unstressed potted plants over stressed potted plants when PGPR consortium was applied with carriers.

In field grown under unstressed condition (Table 4.24), single inoculation of PGPR increased plant height by 6% over plants grown under saline sodic field (Table 4.19). Coinoculation of PGPR increased 3% plant height in unstressed field grown plants. PGPR consortium with carriers increased plant height by 4% in unstressed field grown plants. Similar trend was followed during 2011-2012 (Table 4.25 and Table 4.20)

During 2010-2011, fresh weight of aerial parts (Table 4.22 and Table 4.24) was significantly higher when *Bacillus cereus* was inoculated with maize straw and sugarcane husk (both in pots and field conditions). Coinoculation of both PGPR increased fresh weight significantly by 29% in pots and 44% in field grown plants. Application of PGPR in the form of biofertilizer
increased fresh weight by 34-38% in pots and 55-59% in field grown plants, % increase was higher when sugarcane husk was used as carrier.

The potted plants grown under unstressed condition (Table 4.22) and receiving single inoculation of PGPR with carriers had 10% higher fresh weight over potted plants grown under salt stress (Table 4.17). Coinoculation of PGPR increased 5% greater fresh weight over control under unstressed condition. The % increase over control in fresh weight was 7% in unstressed potted plants over stressed potted plants following the treatment of PGPR consortium with carriers. Similar trend was followed during 2011-2012 (Table 4.23 and Table 4.27).

4.2.17. Effects of biofertilizer on protein contents of leaves under unstressed condition

During 2010-2011 protein content of leaves of potted plants (Table 4.22) was 33-35% higher over control when plants were singly inoculated with *Bacillus cereus* and *Pseudomonas moraviensis* and both type of carrier. Coinoculation of both *Bacillus cereus* and *Pseudomonas moraviensis* significantly increased protein contents by 47% and 39% in pots and field grown plants (Table 4.24) respectively. Application of bioinoculant consortium in the form of biofertilizer increased protein contents by 58-62% in pots grown plants and 75% in field grown plants.

The % increase in protein contents were equally (25%) in field grown under stressed and unstressed condition (Table 4.19 and Table 4.24). Coinoculation of PGPR increased 6% protein in field grown plants under unstressed condition. PGPR consortium with carriers increased protein by 41% in unstressed field over field grown plants under saline sodic field. Similar trend was followed during 2011-2012 (Table 4.20 and Table 4.25).
4.2.19. Effects of biofertilizer on proline contents of leaves under unstressed condition

During 2010-2011 plants grown in pots and field under unstressed condition, proline contents were (Table 4.22 and Table 4.24) significantly higher when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated individually with maize straws and sugarcane husk in pots and field grown plants. However % increase was higher in pots grown plants as compared to field. Coinoculation of both PGPR increased 59% proline contents in leaf of pots grown plants and 41% in field grown plants. Proline contents were significantly (60% and 45%) higher in pots and field grown plants in biofertilizer treatment. Similar trend was followed during 2011-2012 (Table 4.23 and Table 4.24)

4.2.20. Effects of biofertilizer on antioxidant enzymes activities of leaves under unstressed condition

During 2010-2011, superoxide dismutase (SOD) activity (Table 4.22 and Table 4.24) was 39-60 % higher over un-inoculated control both in pots and field grown plants when *Bacillus cereus* and *Pseudomonas moraviensis* were inoculated singly alongwith maize straw and sugarcane husk. Coinoculation of both PGPR increased SOD activity by 79% and 64% in pots and field grown plants respectively. Maximum increase 86% and 80% was observed in pots and field grown plants when consortium of both PGPR was applied alongwith carriers. Similar trend was followed during 2011-2012 (Table 4.23 and Table 4.25).

During 2010-2011 peroxidase (POD) activity (Table 4.22 and Table 4.24) of plants leaves treated with *Pseudomonas moraviensis* and carriers was 40% higher in pots and 25% in field grown plants. *Bacillus cereus* with maize straw and sugarcane husk showed almost equall magnitude (45%) of increase in POD activity. Coinoculation of both PGPR enhanced POD by 70% in pots and 58% in field grown plants. The application of consortium of both PGPR
alongwith carriers exhibited 86% and 66% increases in pots and field grown plants respectively. Similar trend was followed during 2011-2012 (Table 4.23 and Table 4.25).

4.2.2.22. Effects of Biofertilizer on wheat yield under salt stress

During 2010-2011, spike length and number of seeds/spike were significantly higher (Table 4.26) when two PGPR were applied singly along with carriers as well as in coinoculation. Increase in spike length and number of seeds/spike were 21% and 26% higher when PGPR were applied singly along with carriers. Coinoculation of PGPR further increased the spike length and number of seeds/spike by 17% and 20%. Significant higher increase in seed size which was measured as 1000 seed weight was observed in all the treatments over control but *Bacillus cereus* was more effective with both types of carrier material. Maximum increase (26%) was observed when the consortia of PGPR were applied with sugarcane husk as carrier material. Similar trend was followed during 2011-2012.

During 2010-2011 under saline sodic field condition (Table 4.26), PGPR consortium significantly increased (40%) number of plants. This increase was 52% higher over control in the biofertilizer treatments. Significantly higher spike length and number of seeds/spike (were observed when *Pseudomonas moraviensis* and *Bacillus cereus* were coinoculated. Maximum increase (25-35%) was observed in biofertilizer treatment. Biofertilizer treatment significantly increased seed weight by 20% of treated plants. Similar trend was followed during 2011-2012 (Table 4.27).

In field grown under stressed condition, % increase in number of plant/ m² was 8% higher over field grown plants grown under unstressed field (Table 4.29). Coinoculation of PGPR increased 7% plant/ m² in field grown plants under saline sodic condition. PGPR consortium with carriers increased plant/ m² by 26% in saline sodic field over unstressed field grown plants. Similar trend was followed during 2011-2012.
In field grown under stressed condition (Table 4.26), % increase in spike length was 6% higher over field grown plants grown under unstressed field (Table 4.29). PGPR consortium with carriers increased plant/ m$^2$ by 3% in saline sodic field over unstressed field grown plants. The saline sodic field grown plants (Table 4.27) treated with single inoculation of PGPR and carriers increased 5% spike length over potted plants treated with NaCl (Table 4.27). Spike length of the field grown plants was 10% higher over potted plants, following the coinoculation of PGPR and application of PGPR with carriers. Similar trend was followed during 2011-2012 (Table 4.27).

The potted plants grown under unstressed condition (Table 4.28) receiving single inoculation of PGPR with carriers had 7% higher spike length over potted plants grown under salt stress (Table 4.27). Coinoculation of PGPR increased 6% spike length over control in stressed potted plants. The % increase over control in spike length was 11% in stressed potted plants over unstressed potted plants following the treatment of PGPR consortium with carriers.

In field grown under stressed condition (Table 4.26), % increase in seeds / spike was 9% higher over field grown unstressed plants (Table 4.28). Coinoculation of PGPR increased 13% seeds /spike in field grown plants under saline sodic condition. PGPR consortium with carriers increased seeds /spike by 3% in saline sodic field over unstressed field grown plants.

The saline sodic field grown plants (Table 4.27) treated with single inoculation of PGPR with carriers increased 4% seeds/ spike over potted plants treated with NaCl (Table 4.26). Number of seeds / spike of the field grown plants were 11% higher over potted plants, following the coinoculation of PGPR and application of PGPR with carriers.

The potted plants grown under unstressed or unstressed condition (Table 4.26 and Table 4.28) following single inoculation of PGPR with carriers equally improved (28%) seeds / spike over control. Coinoculation of PGPR increased 3% seeds / spike over control in
stressed potted plants. The % increase over control in seeds/spike was 14% in stressed potted plants over unstressed potted plants following the treatment of PGPR consortium with carriers.

Similarly, the field grown plants grown under stressed condition (Table 4.27) exhibited 8% increase in seeds weight over field grown unstressed plants (Table 4.29). Coinoculation of PGPR increased 7% seed weight in saline sodic field grown plants. PGPR consortium with carriers increased seeds weight by 4% in saline sodic field over unstressed field grown plants. The % increase over control in seed weight was 9-10% in potted plants grown under stressed condition (Table 4.26) over potted plants grown under unstressed (Table 4.28) when PGPR were coinoculated or consortium of PGPR was applied with carriers.

Under stressed condition, single inoculation of PGPR with carriers equally increased the seed weight over control in potted and field grown plants (Table 4.26 and Table 4.27). The % increase over control in seed weight was 7% and 16% in stressed potted plants over plants grown under saline sodic field, following the coinoculation and application of PGPR consortium with carriers respectively.

4.2.23. Effects of biofertilizer on wheat yield under unstressed condition

During 2010-2011, number of plant/m² (Table 4.28 and Table 4.29) counted at maturity were 44-60% higher when Bacillus cereus and Pseudomonas moraviensis were mixed with maize straws and sugarcane husk in field grown plants. Coinoculation increased number of plants/m² by 81% and maximum increase 90-97% in plant/m² was observed in biofertilizer treatment. Similar trend was followed during 2011-2012.

Increase in spike length was significantly higher in all the treatments of field grown plants (Table 4.28 and Table 4.29) but in pots grown plants it was only higher in coinoculation+carrier treatments. Spike length was significantly higher when both Bacillus
*cereus* and *Pseudomonas moraviensis* were inoculated with maize straws and sugarcane husk in field grown plants. Coinoculation of both PGPR increased 46% spike length of field grown plants. Maximum increase 37% and 60% was observed in pots and field grown plants in biofertilizer treatment. Similar trend was followed during 2011-2012.

Number of seeds/spike (Table 4.28 and Table 4.29) was significantly higher in field grown plants when *Bacillus cereus* was inoculated with sugarcane husk and maize straws. Single inoculation of *Bacillus cereus* and *Pseudomonas moraviensis* in all other treatment showed non-significant increase with both type of carriers in pots and field grown plants. Coinoculation of both PGPR equally increased number of seeds/spike both in pots and field grown plants. Biofertilizer application increased 55% and 58% number of seeds/spike in pots and field grown plants respectively. Biofertilizer application increased 30% higher seed weight both in pots and field grown plants (Table 4.28 and Table 4.29). Similar trend was followed during 2011-2012.

4.2.24. Cost benefit ratio

The cost economic benefit ratio for the production of per hectare was 1.246 and 1.32 for wheat crop grown in saline sodic field and unstressed field respectively. These results indicate that biofertilizer application may increase the farmer’s benefits by 24% and 42% in salt stress and unstressed condition respectively.

4.3. Discussion

The linear increases in colony count after 72 h of incubation of both *Pseudomonas moraviensis* and *Bacillus cereus*, as observed in the formulated biofertilizer were indicative of favourable growth and multiplication of both the PGPR in the carrier material of the biofertilizer. Greater cfu of the PGPR in the presence of sugarcane husk as carrier material over that of maize straw may be attributed to rich C-source and better moisture holding
capability of sugarcane husk for the growth of PGPR bioinoculants. The coinoculation of *Pseudomonas moraviensis* with *Bacillus cereus* (Fig 4.2) resulted in 60% increase in cfu as compared to single inoculation. In NaCl treated and unstressed soil of potted plants, *B. cereus* and *P. moraviensis* coinoculation in sugarcane husk and maize straw also showed greater cfu over that of single inoculation in the same carrier. Bacillus cereus had greater cfu in stressed soil while in unstressed soil cfu of *P. moraviensis* was higher. The carbon sources are essential for microbiota and survival of PGPR strains depend upon c-source enrichment. The greater cfu is the indicative of carrier induced potential of the PGPR and assisted in proliferation.

*Bacillus cereus* and *Pseudomonas* are capable of solubilizing phorsphorus thus making the P available which has been precipitated and thus unavailable. Noteworthy, these PGPR also have antifungal activity which enabled them to survive and compete with indigenous microflora. Both *Bacillus cereus* and *Pseudomonas fluorescens* exhibit strong antifungal activity against different fungal culture.

PGPR induced decline in EC and SAR of rhizosphere soil of saline sodic field and potted plants grown under stressed condition, and magnitude of decrease was more prominent in potted plants. Greater decrease in is perhaps, due to the sole effects of PGPR in sterilized soil whereas in field interactive effects of chemotatic and edaphic factors modulate the effects and also there is a competition of the PGPR indigenous microflora. Greater decrease in EC and SAR in potted plants may be attributed to the concomitant increases in nutrients like NO$_3$-N, Ca, Mg, K, and P. The observation, that single inoculation with the PGPR had no significant effects on the EC and SAR of rhizosphere soil but coinoculation was effective indicating the synergistic effects between the two PGPR. The higher K/Na and Ca/Na ratio in PGPR treatments were indicative of salt tolerance in plant.
Soil nutrients play important role in the growth and survival of plants under adverse condition (Parewa et al., 2014). PGPR induced reclamation of saline sodic soil which was mediated by decrease in the Na\(^+\), Cl\(^-\) and HCO\(_3^-\) with concomitant increase in P and NO\(_3^-\)N availability. The PGPR are reported to synthesize exopolysaccharides preventing apoplastic flow of Na into the vascular tissue. Application of *Pseudomonas fluorescenes* and *Enterobacter aerogenes* decreased Na contents in maize leaves under salt stress.

The coinoculation of both the PGPR in the presence of maize straw and sugarcane husk increased organic matter, Ca, Mg and K contents in soil of potted and field grown plants under stressed and unstressed conditions. The magnitude of increase was higher in field grown plants and under unstressed condition. The applied sugarcane husk and maize straw are component of compost industry and contained high contents of N, P and K. PGPR along with these carriers were good sources for nutrient availability. Use of *Bacillus subtilis*, *Bacillus megaeorium*, *Acinetobacter baumannii* and *Pantoea agglomerans* as PGPR improved Fe, N, Mn and Zn contents of tomato (*Lycopersicon esculentum* L.) and cucumber (*Cucumis sativus* L.)

Application of PGPR improved the uptake of Mg and Fe in apple leaves. The PGPR are more protected from adverse condition in saline sodic field when they are mixed with carrier. However the positive effect of each PGPR applied singly for improving organic matter of rhizosphere soil was noteworthy in saline sodic soil and in particular in the form of biofertilizer they showed better performance.

The coinoculation of PGPR with carriers were more effective than single inoculation because mixed population of microbiota provides broad spectrum of action with improved efficacy of PGPR strains and combination of different traits. The observed changes in pH, moisture and temperature under salt stress enables PGPR to survive better in mixed inocula over single
inoculation, hence they are more adaptive and competitive to soil environment. Previously inoculation of *Azospirillum*, *Azotobacter*, *Pseudomonas* and *Bacillus* coinoculation proved more effective over single inoculation in improving biomass of *Withania somnifera*. The coinoculation of PGPR was effective for reclamation of salinity over single inoculation.

The observed increase in nutrients contents is correlated with microbial and enzymatic activities and PGPR inoculation further improved the release of nutrients from soil colloidal particles. Nutrient uptake and balancing ability in carrier based PGPR formulation might be attributed to the available nutrients of applied carrier.

The consortium of two PGPR mixed with carriers decreased Na contents of leaves concomitant with (35%) increase in the K uptake in potted and field grown plants under salt stressed condition. Noteworthy, the ability of plants to discriminate between Na\(^+\) and K\(^+\) can be determined by K\(^+\)/Na\(^+\) ratio as assisted by PGPR during present study. In the present study, Na/K and Na/Ca ratio was 30-45% less over control when biofertilizer was applied. Improvement in K, Ca, Mg, and Fe attributed the ability of PGPR in balancing the nutrients. The increase in nutrients uptake of PGPR treated plants is in agreement with previous findings. Previous results also demonstrated that *Pseudomonas fluorescenes* decreased Na contents in maize leaves under salt stress.

The increase in plant height and fresh weight were more prominent in field grown and unstressed plants when compared with potted plant. The observed greater increase in plant height and fresh weight might be attributed to better NO\(_3\), nutrients availability in soil and uptake in treated plants.

Better water holding capacity of the carrier material helped the PGPR to perform efficiently under pots and field condition as evidenced by significant increase in the fresh weight,
chlorophyll, protein and proline contents, of plants treated with carrier based bioinoculant i.e biofertilizer.

In the present study, increase in protein content of biofertilizer treated plant was higher in field grown plants over potted plants grown under unstressed condition. Enhanced protein contents of leaves with subsequent availability of N-source and increased in growth and metabolism might be due to PGPR application. Salt induced degradation/decrease in protein contents of leaves but in the present study PGPR application with carriers decreases the protein degradation over control both in potted and field grown plants by ameliorating salt stress.

The application of biofertilizer increased the proline contents both in pots and field grown plants under salt stressed and unstressed condition. The magnitude of increase was higher in field grown salt stressed plants over potted salt stressed plants. Proline is a protective osmolyte which is related with osmotic adjustment and macromolecules protection during salt stress. Better osmoregulation as evidenced by increase in proline production due to PGPR application appeared as adaptive mechanism under salinity stress.

The application of biofertilizer increased the antioxidant enzymes (SOD, POD) activities in treated plants both in pots and field grown under salt stressed and unstressed condition. The single and coinoculation of PGPR with carriers’ i.e biofertilizer increased SOD and POD activities. The magnitude of increase was higher in field grown salt stressed plants over potted salt stressed plants. Under abiotic stresses, Reactive oxygen species (ROS) are major contributor and act as second messengers. Excessive accumulation of ROS is destructive for cells and detoxification is achieved by enzymic and non-enzymic antioxidant system under such circumstances. The enzymic antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase are highly active against ROS. The observed increase in antioxidant super oxide dismutase (SOD) and peroxidase (POD) in treated wheat leaves
insinuate toward the better adaptability. The observed increase in antioxidant activities may be attributed to the positive role of PGPR on detoxification of reactive oxygen species under salinity stress.

Inoculation of PGPR consortium with carrier materials increased phytohormone production in leaves of reated plants. This effect was more pronounced on IAA and ABA, clearly depicted in IAA/ABA ratio which was higher both in potted and field grown plants grown under salt stressed condition. Many strains of Bacillus and Pseudomonas have been screened for producing IAA. Bacillus cereus and Pseudomonas moraviensis were reported as efficient producers of phytohormones.

Modulation of ABA, IAA and GA level eliminate the deleterious effect of salinity and increase plant adaptability, improves seed germination, root architecture, shoot length and fresh weight. Many microbial strains are capable of producing gibberellin. Among Bacillus species Bacillus pumilus and Bacillus licheniformis, showed strong growth-promoting activity.

Previously inoculation of Bacillus and Pseudomonas improved GA contents of leaves in wheat, rice and other plants. Increased in GA level is correlated with root formation and Bacillus spp are known to improve the endogenous level of GA.

Exposure to salt stress induced a proportional increase in ABA concentration that in most cases is correlated with leaf or soil water potential. Notably, the ABA and GA production was significantly higher in plants raised from seeds treated with biofertilizer. These results demonstrate that the PGPR better proliferate within the carrier materials. Increase in GA and ABA production is correlated with the improvement of stomatal conductance and turgidity. ABA acted as root to shoot stress signal and increase in ABA as result of Pseudomonas moraviensis was accompanied by increase in water uptake and xylem water potential. Increase in ABA contents of leaves might be attributed the ability of PGPR to enhance ABA
flow in xylem and leaves. Higher ABA was found in the shoots of lettuce when *Bacillus subtilis* was applied to lettuce. 

*Bacillus cereus* was more effective for improving spike length and number of seeds/spike. The % increase over control in spike length was greater under unstressed condition and field grown plants, following the treatment of PGPR consortium with carriers. Spike length is a physical component of grain yield. Biofertilizers were previously reported to increase spike length and other yield attributes. *Bacillus cereus* and *Bacillus circulan* resulted marked increase in yield of wheat, maize and Pigeon pea. 

Increase in the seed size measured as weight of thousand seeds might be attributed to PGPR induced indole acetic acid (IAA) production and better nitrogen availability. Application of biofertilizer induced increase in yield attributes over control and magnitude of increase was higher under unstressed condition and field grown plants over stressed and potted plants. Increase in yield of biofertilizer treated plants is correlated with the better survival efficiency of PGPR in carrier material. The substantial increase in grain yield and 1000 grain weight is in agreement with previous findings where biofertilization showed marked increase in yield components of cereals. 

**Conclusions**

It is inferred from the present findings that the PGPR proliferate better and augmented the shelf life of the PGPR in the presence of carriers which may seems to protect the PGPR from desiccations stresss but also provide C/N source as they are rich in organic matter. *Bacillus cereus* strain was more suitable for soil reclamation and alleviation possibly due to its better survival. Sugarcane husk serve as suitable carrier. The presence of carrier material along with consortium of PGPR, acting synergistically appears better solution to combat salinity stress. Application of PGPR consortium imparted positive effects on physiological and biochemical parameters. The effects were significant both on soil health as well as on the plant
productivity. The said biofertilizer was also effective under unstressed condition both in field and pots. Under unstressed condition plants treated with carrier based biofertilizer accumulated higher nutrients, protein contents and increased yield attributed over salt stressed plants.

Chapter - 5

5. Introduction

Agrochemicals are proposed to manage agricultural ecosystem and the community of organisms of a farming area. Agrochemicals include broad range of products but importantly categorized by: (1) fertilizers (2) soil conditioners (3) liming and acidifying agents (4) pesticides, and (5) chemicals used in animal husbandry (antibiotics and hormones). The adequate, safe food supply cannot be acquired without using agrochemicals.

Effects of polyamines on different crops is a current topic in physiology, but unfortunately most of these researches were limited and conducted in controlled environments or controlled environment. Polyamines are organic compounds with two or more amino groups in their structure. Plants polyamine occur in actively growing tissues and are synthesized under stressed conditions in which they have pivotal role in cell division, root formation, fruit setting and ripening, defense mechanism, and embryogenesis. Putrescine not only involved in stabilizing effects by binding to the intracellular anions (chromatin, proteins, DNA and RNA), it also contributes to several regulatory functions. Many physiological processes, such as organogenesis, embryogenesis, floral initiation and development, leaf senescence, fruit development and ripening, and abiotic and biotic plant stress responses.

In higher plants tolerance against salt stress is coped by polyamines. In case of salinity, polyamines metabolism depend on plant species, plant system, and duration of salinity exposure. Polyamines are synthesized by precursor S-Adenosyl methionine (SAM) which is also precursor of ethylene synthesis. Ethylene biosynthesis is blocked by polyamines because
they inhibit the conversion of $S$-Adenosyl methionine (SAM) into 1-aminocyclopropane-1-carboxylic acid (ACC), which is converted into ethylene. Hence polyamine and ethylene regulate each other either directly or by competition for SAM. Putrescine is synthesized by enzyme arginine decarboxylase ADC. ADC is controlled by two genes $ADC1$ and $ADC2$ in case of *Arabidopsis thaliana* L.

Several mechanisms have been defined for the activity of putrescine under salt stress. Putrescine is linked with $H_2O_2$, ROS and NO signaling produced during abiotic stresses and known to induce salt tolerance in plants. Putrescine and other polyamines, $H_2O_2$ and NO act synergistically with stress phytohormone ABA to regulate stomatal conductance.

Putresine is involved in $Ca^+$ and $K^+$ homeostasis under stress conditions. The increase in cytoplasmic $Ca^+$ halted $Na^+/K^+$ entry in cytoplasm, which results suppression of $Na^+/K^+$ release from the vacuole. The higher influx and accumulation of $Na^+/K^+$ provide tolerance to abiotic stresses.

Being positively charged moieties putrescine and polyamines interact easily with negatively charged proteins. The increase concentration of putrescine under abiotic stresses blocked the ion channel especially fast-activating vacuolar (FV) cation channel in a charge-dependent manner. Phosphorylation and dephosphorylation of ion channel proteins by affecting protein kinase and phosphatase activities contributes to regulation of ion channels.

This study reports the effects of putrescine foliar spray on alleviation of osmotic stresses in plants by increasing growth, physiology, and yield of wheat under salt stressed or unstressed and pot or field conditions. The emphasis was made on wheat physiology, nutrients accumulation and yield.

5.1. Material and methods
5.1.1. Plant material and growth condition

Field Experiment

Seeds of *Triticum aestivum* L. cv. Inqlab 91 were sown in saline-sodic soil at Soil salinity Research Institute Pindibhatian (maximum average temperature = 23°C, rainfall = 7 mm and relative humidity = 60%) and in un-stressed soil of Quaid-i-Azam University (maximum average temperature = 22°C, rainfall = 9 mm and relative humidity = 67%) Islamabad. Aqueous solution of putrescine at 0.241g/L was applied on plants of fields at 14 and 30 days after seed germination. Plants sampling was done at early vegetative stage 57 days after sowing (DAS) for physiological parameters and at 159 DAS maturity for yield parameters.

Pot Experiment

Pot experiment was conducted in wire house under natural condition at Quaid-i-azam University Islamabad (maximum average temperature = 22°C, rainfall = 9 mm and relative humidity = 67%). The aqueous solution of NaCl was added to the potted (autoclaved) soil at the rate of 150 mM in irrigation water after 7 and 14 days of seed germination to maintain EC = 3.7 dSm\(^{-1}\) and compared with un-stressed condition (EC= 0.30 dSm\(^{-1}\)). Aqueous solution of putrescine at 0.241g/L was applied to potted plants twice at 14 and 30 days after seed germination. Plants sampling was done at early vegetative stage (57 days after sowing) for physiological parameters and at maturity for yield parameters.

5.1.4. Determination of free putrescine

Extraction for free putrescine from plants leaves was done by the procedure. The plants leaves were extracted in 5% cold HClO\(_4\) with ratio of 100mg/ml HClO\(_4\). After keeping on ice bath for 1 h, samples were centrifuged at 48000 rpm for 20 min and supernatant containing free polyamines were stored at -20°C for further analysis. The benzoylation of standard and
plant extracts were made according to Redmond and Tseng 1979. In 500 µl, 1 ml of 2N NaOH was added along with 10 µl of benzoyal chloride. Samples were vortexed and incubated for 20 min at room temperature. Benzoyal polyamines were extracted in 2 ml diethyl ether and centrifuged at 1500 rpm for 5 min. The ether phase (1 ml) was collected and dried using rotary thin film evaporator (RTE) and redissolved in 100 ml methanol (HPLC grade). For standard, 50 µl of putrescine ( ) was used. The analysis was made on HPLC (Shimadzu, C-R4A Chromatopac; SCL-6B system controller) using UV detector and C-18 column (39x300mm) for identification of putrescine. Prior to injection in the column, the sample (20 µl) was filtered through 0.45 millipore filter. Acetonitrile: water (52:48, v/v) was used and flow rate (1 ml/min) was adjusted for an average run of 20 min/sample. Pure putrescine (sigma, USA) was used as standard and putrescine was identified on the basis of retention time and peak area at 254 nm.

5.2. Results

5.2.1. Effects of putrescine foliar spray on leave nutrients

During 2010-2011, putrescine foliar spray significantly decreased Na (Table. 5.1) accumulation by 64% in the leaves of the plants, grown under salt stress both in pot experiment and field experiment. The percent decrease in Na was 30% higher in the pots plants grown plants over the field grown plant. Leaves Na content was decreased by 64% and 34% over control in potted and field grown plants respectively when grown under salt. No significant decrease in Na accumulation was observed in plants grown under unstressed condition.

Putrescine foliar spray enhanced K accumulation by 21% and 19% over control, in potted plants grown under stressed or unstressed condition. Similarly, K contents were 17% and 41% higher in saline sodic field and unstressed field grown plants respectively. K
accumulation in the leaves of putrescine treated plant was equally increased (20%) over control in potted plants grown under stressed or unstressed condition. Increase in K content was 4% higher in potted plants over field grown plant, grown under stressed condition while under unstressed condition 30% greater K was observed in field grown plants over potted plants.

Significantly higher (35% and 39%) Ca contents were observed in the leaves of putrescine treated plants grown in pots or field, under stressed condition. Under unstressed condition, increase in Ca content was 30% over control only in field grown plants. Similarly, potted plants grown under salt stress had 19% higher Ca than potted plants grown under unstressed condition. Plants grown under unstressed field condition accumulated 9% greater Ca over plants grown under saline sodic field condition. Plants grown under saline sodic field condition and unstressed field accumulated 8% and 17% greater Ca over potted plants grown under salt stress and unstressed condition respectively.

Mg contents of putrescine treated plants were 25% and 57% higher over control in potted plant grown under stressed and unstressed condition respectively. Plants grown in saline sodic field and treated with putrescine also accumulated 16% higher Mg over control. Potted plants grown under stressed condition accumulated 33% greater Mg over potted plants grown under unstressed condition. Similarly, plants grown under saline sodic field condition had 5% greater Mg over plants grown under unstressed field condition. Increase in K content was 41% in potted plants grown under stressed condition over plants grown under saline sodic field. Under unstressed condition 13% higher Mg was observed in field grown plants over potted plants.
Among all putrescine treated plants, the accumulation of Ca and Mg and K was higher in plants grown under saline sodic field condition while decrease in Na was greater in pots grown plants provided with 150 mM NaCl. Similar trend was followed during 2011-2012.

5.2.2. Effects of putrescine foliar spray on wheat growth

During 2010-2011, putrescine treatment increased the plant height by 26% and 29% over untreated control in saline sodic and unstressed field respectively (Table 5.3). Putrescine treatment to plants grown under axenic condition, revealed 23% and 20% increase over control using single salt (NaCl) and unstressed pots respectively (Table 5.2). Putrescine treated field grown plants under stressed and unstressed had 7% and 15% higher plant height respectively as compared to potted plants grown under stressed and unstressed condition. Putrescine foliar spray equally contributed in improving plant height over control of potted plant grown under stressed or unstressed condition. However % increase in field grown plants was higher under unstressed condition.

The fresh weight (Tables 5.3) of the aerial parts (single plant) was 89% greater in putrescine treatment for plants grown in saline sodic soil of Pindibhatian. Putrescine sprayed potted plants grown under salt stress exhibited 20% greater fresh weight over control. Unstressed field grown putrescine treated plants, 84% greater fresh weight was observed over control. Putrescine treatment to field grown plants increased 69% fresh weight of field grown plant in saline sodic field over potted plants grown under salt stress. Similarly, % increase in fresh weight of field grown potted plants was 80% over potted plants grown in sterile soil. Similar trend was followed during 2011-2012.

5.2.3. Effects of putrescine foliar spray on wheat physiology
During 2010-2011, the leaves of the plants grown in saline sodic field or unstressed condition, produced 14% greater chlorophyll over control (Tables 5.3). Potted plants under unstressed condition also exhibited 22% higher chlorophyll in putrescine sprayed plants over control (Table 5.2). Putrescine foliar spray exhibited 11% higher chlorophyll contents in saline sodic field over potted plants grown under stressed condition. However the % increase under unstressed condition was 15% higher in field grown plants. Similar trend was followed during 2011-2012.

During 2010-2011, the accumulation of protein contents was 33% and 42% higher in field grown plants (Table 5.3) as compared to potted plants grown under stressed or unstressed condition respectively (Table 5.2). The significantly higher (38%) protein contents were recorded in putrescine treated plants, grown in saline sodic field over control. There was 6% higher protein content in potted plants grown under unstressed condition over potted plants grown under stressed condition. Percent increase in unstressed field grown plants was 15% higher over saline sodic field grown plants. Similar trend was followed during 2011-2012.

The sugar content (Tables 5.2) of putrescine foliar sprayed plant was 19% higher in stressed potted plant over control. Potted plants grown under unstressed and stressed condition treated with putrescine foliar spray exhibited equally 10% increase over control. Similarly potted plants grown under stressed condition had 7% higher sugar content over plants grown under saline sodic condition. There was no significant difference in putrescine treated plants over control, in potted plants grown stressed or unstressed, and field grown plants under unstressed condition. Similar trend was followed during 2011-2012.

During 2010-2011, the proline contents of leaves (Tables 5.3 and 5.2) were 97% and 90% higher over control in putrescine treatment, in saline sodic field and salt stressed pots. The % increase was higher (7%) in the field. In unpotted plants grown under salt stress, the effect of
putrescine was less pronounced (53%) than potted plants grown under salt stress. Proline content of treated plants was 37% and 42% higher over control in un-potted plants grown under salt stress and field grown plants. Under unstressed condition % increase was 5% higher in field grown plants over potted plants. Similar trend was followed during 2011-2012.

During 2010-2011, putrescine foliar application strengthens the endogenous level of free putrescine (Tables 5.2 and 5.3) in stressed and unstressed leaves of plants grown in pots and field condition. % increase was higher in field condition where 45% higher free putrescine was observed both in stressed and un-stressed condition over control. The plants grown in pots or field under unstressed condition exhibited higher putrescine in the leaves than stressed plants. In pots grown plants 42% and 31% higher putrescine was observed in unstressed and stressed condition respectively. Similarly, increase in endogenous level of putrescine was 82% and 75% higher in saline sodic and unstressed field respectively. Under stressed conditions, field grown plants exhibited 47% higher endogenous putrescine over pots grown plants. Field grown plants under unstressed condition had 4% greater putrescine over field grown plants grown under saline sodic field. In potted plants 36% higher putrescine was observed under unstressed condition over stressed condition. Similar trend was followed during 2011-2012.

During 2010-2011, superoxide dismutase (SOD) activity of field grown plants (Table 5.3) was higher than that of pots grown plants (Table 5.2) both under unstressed or salt stressed condition. Similar pattern of response was exhibited by putrescine treatment. The magnitude of response was 70% and 74% higher in plants grown in saline sodic field and salt stress respectively. The increase in SOD activity of putrescine treated plants under unstressed condition was 28% and 42% greater over control in field and pots grown plants respectively.
Potted plants grown under stressed condition had 27% less SOD activity than plants grown under saline sodic condition. Putrescine treated plants exhibited equal increase over control in field grown plants grown under stressed or unstressed condition. In potted plants grown under stressed condition, SOD activity was 44% higher over potted plants grown under unstressed condition.

Putrescine foliar spray increased the peroxidase (POD) activity of leaves by 40% and 92% over control in pots and field grown plants respectively, under salt stress condition. This increase was non-significant in pots or field grown plants under unstressed condition. Potted plants grown under stressed condition had 51% less POD activity than plants grown under saline sodic condition. Putrescine treated plants exhibited 32% increase over control in field grown plants under stressed condition over field grown plants under unstressed condition. In potted plants grown under stressed condition, POD activity was 26% higher over potted plants grown under unstressed condition. Similar trend was followed during 2011-2012.

During 2010-2011, putrescine foliar spray effectively increased endogenous phytohormones indole acetic acid (IAA), abscisic acid (ABA) and gibberellic acid (GA) as compared to untreated control in all treatments (Tables 5.2 and 5.3). Increase in IAA and GA contents was 6-10% higher in unstressed condition both in pots and field grown plants over stressed plants. Field grown plants exhibited 10-25% higher phytohormones in leaves than pots grown plants. IAA contents of putrescine treated plants were 46% and 39% in pots grown plants under stress and unstressed condition respectively. This increase was 64% and 75% in field grown plants respectively. Gibberellic acid (GA) contents 40-50% higher in putrescine treated plants over control in pots or field grown plants, under stress or unstressed condition. Under stress condition 10% higher IAA and 20% higher GA contents were observed in plants grown under saline sodic condition than potted stressed plants.
The abscisic acid (ABA) contents were 100% higher in plants grown under stressed condition over that of unstressed plants. ABA contents were 60% and 74% higher in potted and field grown plants under stress condition (Table 5.2 and Table 5.3), while 30% higher ABA was observed over control in putrescine treated plants, both in pots and field grown plans under unstressed condition.

Putrescine treated plants exhibited 13% increase in ABA over control in field grown plants under stressed condition over potted plants. In potted plants grown under stressed condition (Table 5.2), ABA content was 30% higher over potted plants grown under unstressed condition. In field grown plants (Table 5.3), 41% higher ABA was observed under stressed condition over unstressed condition. Similar trend was followed during 2011-2012.

Chapter - 6

6. Introduction

Bacteria residing in the rhizosphere influence growth of host plants by modulating endogenous level of phytohormones. The auxin or indoleacetic acid (IAA) is of wide occurrence among PGPR. In phytopathogenic bacteria, such as *Pseudomonas syringae*, IAA is produced from tryptophan. IAA predominantly synthesized by these PGPR via tryptophan-dependant pathway, through indolepyruvic acid or alternate pathways using indoleacetamide.

The salt tolerance potential, phosphate solublization, IAA production and greater antifungal activity render *Pseudomonas moraviensis* as strong bioinoculant under salt stress.

Biosynthesis of IAA from tryptophan is a five step process which is encoded by trp genes. Five different pathways are described for IAA synthesis starting from tryptophan. The knockout studies reveal that some microorganisms follow more than one pathway. It was evident in some microbes that IAA production was not terminated even when single pathway was blocked.
Some pathogenic bacteria follow indole-3-acetamide pathway. In this pathway, tryptophan monooxygenase (encoded by iaa M gene) converts tryptophan to IAM and then IAM is hydrolyzed to IAA and ammonia by an IAM hydrolase (encoded by iaa H gene). *Agrobacterium tumefaciens, P. savastanoi, Pseudomonas syringae* and *Pantoea agglomerans* follow this pathway.

*P. agglomerans, Azospirillum, Bacillus, Bradyrhizobium, Enterobacter cloacae, Paenibacillus, Pseudomonas, Rhizobium* and cyanobacteria followed a different pathway in which tryptophan is transaminated to indole-3-pyruvic acid (IPA). This IPA is decarboxylated to indole-3-acetaldehyde (IAAld), which is then oxidized to IAA by a dehydrogenase. The decarboxylation is catalysed by enzyme indole-3-pyruvate decarboxylase (encoded by ipdC gene).

In tryptamine pathway, tryptamine is decarboxylated into tryptophan which is converted to IAAld by an amine oxidase. In *Agrobacterium* and *Rhizobium* spp, indole-3-acetonitrile (IAN) is converted into IAM which is finally converted into IAA.

Transposable elements (TEs) are DNA sequences, moveable from one location to other in the genome. These elements are of wide occurrence both in prokaryotes and eukaryotes. In some cases gene inactivation, expression or recombination is promoted by TE mobilization (Muñoz-López and García-Pérez, 2010). Transposons are formed by two different pathways.

1) By the replication of a new copy of the element into the target site, leaving nothing at the original site. 2) By the excision of the element from the original site followed by reintegration into a new site. Some transposon in bacteria replicates via DNA intermediates while other (mostly mobile) elements transpose through RNA intermediates.

Transposon mutagenesis has previously been used to construct transposon insertion mutant library for screening of mutants. Activity of TEs (Transposan Elements) can be modified
positively or negatively. Role of TEs in the reorganization and internal deletion of genomic DNA is also very important tool used in molecular studies.

Transposon mutagenesis of Agrobacterium tumefaciens by interruption of a putative oligoketide cyclase/lipid transport protein resulted reduced IAA synthesis in constructed mutants. Similarly insertion mutagenesis by disrupting ipdc gene that encodes indolepyruvate decarboxylase, in plant growth-promoting bacterium Pseudomonas putida GR12-2 also result loss of IAA production in IAA-deficient mutants.

Counter selection of E. coli on minimal media was major limitation in biparental mating. The construction of hem A glutamyl tRNA reductase knock out mutant requiring 5, aminolevulinic acid (ALA) can counter this limitation. The hem A gene encoded NAD (P) H-dependent glutamyl-tRNA reductase of the C5 pathway for 5-aminolevulinic acid (ALA) synthesis. This hem A is thus essential for electron transport complexes and for various enzymes and proteins.

The δ-Aminolevulinic acid (ALA) is key biosynthetic precursor of tetrapyrroles. In bacteria it is synthesized from glutamate which ligates to tRNA (Glu), reduced to glutamate-1-semialdehyde (GSA) and finally converted to ALA. The tetrapyrroles pigments for growth cannot be synthesized in the absence of glutamyl tRNA reductase (hem A).

Pseudomonas moraviensis wild type strain has ability to synthesize IAA and addition of tryptophan to culture media increased the IAA synthesis. To determine the affectivity and ability of Pseudomonas moraviensis for conversion of tryptophan to IAA, transposon mutagenesis was used to construct transposon insertion mutant library of IAA deficient mutants. The transposon mutants were screened out on the basis of tryptophan conversion to IAA.

6.2. Materials and methods
Plant Growth Promoting Bacteria (PGPR) *Pseudomonas moraviensis* and *Bacillus cereus* were grown on LB media (10% Tryptone, 5% yeast extract and 5% NaCl pH 7.4) and isolation of DNA was done by using Ez-10 spin column genomic DNA kit according to instruction of manufacturer (Bio basic Inc. Ontario Canada). *E. coli* strain was also grown on LB medium supplemented with 50 µg/ml kanamycin and 100 µg/ml ampicillin. Isolated DNA of all strains was amplified by using T-7(TAATACGACTCACTATAGGG) forward universal primers and M-13(AGCGGATAACAATTTCACACAGGA) reverse primer. The initial denaturation was done at 94°C for 1 min followed by 35 cycles of temperature profile: denaturation at 94°C for 20 sec, annealing at 45°C for 60 sec, extension at 72°C for 180 seconds plus one additional cycle of chain elongation at 72°C for 10 min under following optimizing condition.

Amplified PCR products were purified by EZ-10 spin column using PCR purification kit (Bio basic Inc. Ontario Canada) and run at 90 V for 1 h on 1% agarose gel and visualized under UV-transilluminator (Safe Imager™ 2.0 Blue Light Transilluminator).

### 6.2.1. Cloning and Expression Vector

The conventional protocol was used for recombinant DNA techniques. The amplified DNA was cloned by using TA cloning kit into topo cloning vector pCR 2.1 vector and transformed into *E. coli* XL1. Selected constructs were sequenced by Quintarabio University of California.

Sequenced data were analyzed using BLAST-Basic local alignment search tools program through the network service of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

### 6.2.2. Isolation of plasmid DNA
All bacterial strains were grown in LB medium containing 50 µg/ml ampicillin and 50µg/ml Kanamycin. Overnight culture was used for isolation of plasmid DNA and extraction was done by using EZ-10 spin column plasmid DNA Minipreps Kit and examined on 1% agarose gel. Isolated plasmids were sequenced by Quintarabio University of California. Sequenced data were analyzed using BLAST-Basic local alignment search tools program.

6.2.3. Bacteria and plasmids

Two, *E. coli* donor strains (E. coli S17 pir D hemA (ST 18) and its parent strain, *E. coli* S171 pir) and recipient *Pseudomonas moraviensis* were used for mating experiments (Table 1).
6.2. 4. Isolation of Plasmid DNA

Bacterial strain was grown in LB containing 50 µg /ml ampicillin and 50 µg /ml Kanamycin. Overnight culture was used for isolation of plasmid DNA and extraction was done by using EZ-10 spin column plasmid DNA Minipreps Kit (Bio Basic Toronto Canada) and examined on 1% agarose gel. Isolated plasmids were sequenced by Quintarabio University of California. Sequenced data were analyzed using BLAST-Basic local alignment search tools program.

6.2.5. Media and growth conditions

E. coli mutants and transformants (*Pseudomonas moraviensis*), were grown in, Luria–Bertani (LB) medium supplemented with kanamycin (25 mg mL\(^{-1}\)), ampicillin (10 mg mg mL\(^{-1}\)), chloramphenicol (34 mg mL\(^{-1}\)) and tetracycline (10 mg mL\(^{-1}\)). Transformed *Pseudomonas* was selected by using LB or modified AB minimal medium. Minimal medium was supplemented with 60 mg mL\(^{-1}\) gentamicin or 20 mg mL\(^{-1}\) tetracycline.

Wild-type E. coli S17l pir and the mutant E. coli ST18 were cultured overnight in 5 mL LB broth supplemented with 50 mg mL\(^{-1}\) ALA (5-aminolevulinic acid).

6.2.6. Construction of the hemA knockout in

a) E. coli S17k pir

Chromosomal genes were disrupted for the generation of *E. coli* S17l pir D hemA (ST18) knock out mutant as by replacing the gene of interest with a kanamycin resistance (kan) gene.
6.2.7. Mating procedure

Broad host range plasmid (pLAFR3) was used for mating. The tetracycline resistance cassette carried by vector was introduced into parent (S171 pir) and donor strain (ST18) (Sambrook et al., 1989). The cultures of donor and recipient were incubated overnight at 30 - 37 °C in shaker (Gerhadt Rotoshake LS 500) at 200 rpm using LB broth + tetracycline or ALA (5-aminolevulinic acid).

24 h old culture (200 mL) of the recipient and 2 mL of the donor strain were centrifuged for 1 min at 16 000 g. Pellets were suspended in 50 mL LB broth and mixed well. Agar plates containing the dropped suspension were incubated for 6 h at 30 or 37 °C. The cells were scraped off the agar plate after incubation and suspended in 1 mL LB broth.

The mating efficiency was determined from the serial dilutions of a mating experiment which was plated on agar plates under the conditions appropriate for selection against the donor (with or without tetracycline) for determining the ratio in percent between the total number of recipient and donor strains containing the pLAFR3 plasmid.

6.2.8. Selection against E. coli S17 lpir and E. coli ST18

The AB minimal medium comprising tetracycline (Tc) and glucose was used as plating material for *Pseudomonas*.

The deleted hem A gene and ALA (5-aminolevulinic acid) were required for ST 18 growth on culture media. The recipient strain containing transformed plasmid can be grown with appropriate antibiotic like tetracycline. Successful mating efficiency of Tc resistant *Pseudomonas* was verified by culturing and recovery of pLAFR3 was made by plasmid preparation.

6.2.9. Transposon mutagenesis

The recipient (*Pseudomonas moraviensis*) and donor strains of *E. coli* (S171 pir and ST18) containing pBT20 were scrapped from overnight incubated plates suspended in 500 mL LB...
broth. The donor strains and recipient strain were mixed in 1:1 ratio, spotted on agar plates and incubated for 2 h. The scraped off mating was re-suspended in 2 mL LB broth. The number of mutants was determined by growing on LB agar supplemented with Gentamicin (E. coli ST18), and chloramphenicol used for selecting against E. coli S171 pir. About 1000 clones were obtained for each donor and identification of auxotrophic mutants for tryptophan was made on LB plates to AB minimal medium plates supplemented with Gentamicin.

6.2.10. Quantification of IAA production

Wild-type and IAA-deficient Pseudomonas moraviensis screened as mutants were propagated overnight in 5 ml of DF (Dworkin Foster salts minimal media), and then 20 µl aliquots were transferred into 5 ml of DF salts minimal media supplemented with 100 g/ml of L-tryptophan. After incubation for 42 h, the density of each culture was measured by spectrophotometer at 580 nm, and the bacterial cells were removed from the culture medium by centrifugation (5,500 rpm, 10 min). An aliquot of the supernatant(1 ml) was mixed vigorously with 4 ml of Salkowski’s reagent (150 ml of concentrated H₂SO₄, 250 ml of distilled H₂O, 7.5 ml of 0.5 M FeCl₃·6H₂O (Gordon and Weber 1951) and allowed to stand at room temperature for 20 min, the absorbance was measured at 535 nm on a spectrophotometer. The concentration of IAA in each culture medium was determined by comparison with a standard curve prepared by 5-100 µg ml⁻¹ of IAA (Sigma Aldrich USA).
6.3. Results

6.3.1. Resistance of *Pseudomonas moraviensis* against antibiotics

*Pseudomonas moraviensis* strain was found to be resistance against Chloramphinicol, Ampicillin, Hygromycin, and Penicillin while no resistance was shown against Gentamycin, tetracycline, kanamycin and streptomycin.

Seven different plasmids enlisted in table 2 were used for transposon mutagenesis. Among these plasmids POT 182, PMH 1801 and PJQ 118 were able to produce mutants and highest number of mutants were determined from POT 182.

The mutant(s) that grew normally in the presence of L-tryptophan but grew slowly (24-72 h) or failed to grow in the presence of L-tryptophan were considered as putative tryptophan sensitive mutants. Most of the transconjugants grew within 24-48 h as wild type *Pseudomonas moraviensis* grows. However some colonies failed to grow even after 10 days, and some colonies grew poorly in the absence of tryptophan.

Results of transposons mutagenesis revealed that *E.coli* strain ST-18 and S17λ, pir were transformed with suicide plasmid. In transposon mutagenesis 1.8x10⁶ transposons were determined and 28000 mutants were obtained for *Pseudomonas moraviensis*. Similarly one hundred and four (104) auxotroph mutants for tryptophan conversion were identified for *Pseudomonas moraviensis*.

6.3.2. Quantification of IAA production

Production of IAA in wild type strain of *Pseudomonas moraviensis* was higher than mutant strain in the absence of tryptophan. The increase in IAA concentration was significant when tryptophan was added in culture media and wild type *Pseudomonas moraviensis* respond efficiently by producing higher IAA as measured in 42 h old culture. IAA production increased greatly with increase in the concentration of tryptophan added to wild type strain, while a slight increase was observed in mutant strains at 100, 200 and 400 µg/ml. The highest
amount of IAA $81.3 \pm 2.55$ was produced by wild type strain when 400 µg/ml tryptophan was added, while mutant strains showed $1.8 \pm 0.16$ IAA at same concentration.

### 6.4. Discussion

The *Pseudomonas moraviensis* strain genome has not been sequenced and so far no data is available on mutants of this strain. The initial characterization by transposon mutagenesis indicated that the gene inactivated by the transposon insertion in the mutants is homologous to the *ipdc* gene. The *ipdc* encodes indolepyruvate decarboxylase, a key enzyme in the indolepyruvic acid pathway for conversion of tryptophan into IAA.

The POT-182 is a mobilizable suicide plasmid and *E. coli* S17-1 contains an integrated derivative of RP4 and allows conjugative transfer of the vector. Trans conjugants are identified by resistance to tetracycline. The adjacent fragments to the transposes are cloned following digestion with Bam HI, ClaI, EcoRI or SacI and re-ligated. The re-ligated vectors are easily transformed and propagated in any suitable host (*E. coli* like ST18).

The hem A deleted mutant (*E. coli* ST 18) used in present study is suitable donor for *Pseudomonas* spp and also imperative of transposon generation when *mariner* transposon (which are transmitted horizontally), were used on mobilized suicide plasmid like POT-182. Transposon mutagenesis by pLAFR3 *Pseudomonas moraviensis* reveals the stable transposons formation for studying mutagenesis in this bacterium. The frequency of the tryptophan auxotroph mutants was high with 28000 transconjugants.

The results of generation time of *E. coli* S17λ coincide with previous findings Results revealed that mating efficiency depends upon recipient strain as described previously. The data obtained in transposons mutagenesis and determination of auxotroph mutants corroborate with the findings.

The greater increase in IAA production in response to tryptophan addition as measured by ($\mu$g/ml/OD$_{600}$ unit)$^a$ reveals the effectiveness of wild type strains. The potential of
Pseudomonas moraviensis for the production of IAA. Tryptophan being precursor of IAA is beneficial and many strains of Pseudomonas are capable of producing IAA in the presence of tryptophan. Slight increase in IAA concentration was observed in the media containing mutants and tryptophan at 200 µg /ml and 400 µg /ml. The possible increase in IAA in mutant containing media is indicative of indolepyruvic acid presence which becomes reactive in Salkowski’s reagent.

The addition of ALA (5-aminolevulinic acid) complemented this mutation and allows the E-coli growth on media. This hem A deleted mutant (E-coli ST 18) was suitable donor for Pseudomonas spp and also imperative of transposon generation when mariner transposon were used on mobilized suicide plasmid. The mariner family transposon shows relatively low target site and wide range of hosts both from prokaryotes and eukaryotes.

6.5 Conclusions

Pseudomonas moraviensis wild type strain used in the present study has the ability to produce IAA and this effect was further stimulated in the presence of IAA precursor tryptophan. The hem A deleted mutant (E-coli ST 18) used in present study proved to be suitable donor for Pseudomonas moraviensis as it has already been used for Pseudomonas Spp. ST 18 roles seems to be imperative of transposon generation in the presence of mariner transposon on mobilized suicide plasmid POT-182 and pLAFR3. In transposon mutagenesis, 28000 mutants were obtained for Pseudomonas moraviensis, out of which one hundred and four (104) auxotroph mutants for tryptophan conversion were identified. Possibly transposon insertion has disrupted ipdc which encodes for indolepyruvate decarboxylase, a key enzyme in the indolepyruvic acid pathway for conversion of tryptophan into IAA. Screening of IAA deficient mutant revealed that highest amount of IAA was produced by wild type strain at 400 µg /ml tryptophan addition, while most of the auxotroph mutants were failed to produce IAA in Salkowski’s reagent at same concentration. Further screening of auxotroph mutants
on molecular level and application on selected plants is necessary to understand the effectiveness of IAA deficient mutants.
5.2.4. Effects of putrescine foliar spray on wheat yield

During 2010-2011, the length of spike (Table 5.4) was lower in plants of saline sodic soil of the field than that of unstressed field. There is was no significant effect of putrescine under saline sodic condition. No significant effect of putrescine was recorded in potted plants under stressed and unstressed condition (Table 5.4).

Under stressed conditions, field grown plants exhibited 20% greater spike length over potted plants. Field grown plants under unstressed condition (Table 5.4) had 5% greater spike length over field grown plants grown under saline sodic field. In potted plants (Table 5.4), 18% higher spike length was observed under unstressed condition over stressed condition.

Higher number of seeds was produced by plants of saline sodic soil as compared to that of unstressed field irrespective of putrescine treatment. The plants in the pots produced less seeds though putrescine showed stimulation. It is obvious that 30% increase in seed yield can be obtained with putrescine. Field grown plants under unstressed condition (Table 5.4) had 10% greater seeds over field grown plants, grown under saline sodic field. In potted plants (Table 5.4), 6% higher seed number was observed under unstressed condition over stressed condition.

The seed size as measured by thousand seed weight was significantly greater in putrescine treatment both under unstressed and stressed condition in pots and fields (Table 5.4). The 8% increase was higher under unstressed condition in field. Under stressed conditions, field grown plants exhibited 6% higher seed weight over potted plants. Field grown plants under unstressed condition had 17% greater seed weight over field grown plants grown under saline sodic field. In potted plants 9% higher seed weight was observed under unstressed condition over stressed condition. Similar trend was followed during 2011-2012 (Table 5.4).
5.2. 5. Cost benefit ratio analysis

The cost economic benefit ratio for the production of per hectare was 1.08 and 1.16 for wheat crop grown in saline sodic field and unstressed field respectively. These results indicate that biofertilizer application may increase the farmer’s benefits by 8% and 16% in salt stress and unstressed condition respectively.

5.3. Discussion

Though the stimulatory effects of putrescine on wheat were less than tryptophan addition with PGPR (chapter #2 ) and carrier based biofertilizer (chapter # 4), but putrescine application had been proved better than single inoculation of PGPR specifically for physiological parameters. Decrease in Na due to putrescine foliar application, might be attributed to the pivotal role of putrescine in stabilizing membrane and maintaining cation-anion balance. Increase in NO$_3$-N, P and K accumulation in putrescine-treated leaves is in harmony with previous results. The effects of amino acid glycine or lysine treatment, on improving NO$_3$-N, P and K accumulation in leaves have also been reported. Increase in Ca content of putrescine treated leaves insinuates toward the increasing adoptability of plants in maintaining turgor under salinity.

The higher fresh weight of aerial parts in plants grown under saline-sodic field as compared to that of saline soil of pots may be attributed to the less osmotic effect of salinity combined with sodicity. Under salt stress, endogeneous level of putrescine declines, and in such cases exogenously applied putrescine helps to maintain the threshold level to cope with oxidative stress. Higher plant height and fresh weight of garden Dahlia (Dahlia pinnata L.), treated with different concentration of putrescine.
Chlorophyll production due to salinity and sodicity and ameliorative effects of putrescine was less in saline sodic soil than in the soil with induced salinity. Decrease in chlorophyll under salt stress is associated with 5-aminolaevulinic acid degradation, which is a precursor of chlorophyll. Increase in chlorophyll of putrescine-treated leaves documented in Madagascar periwinkle (*Catharanthus roseus* L.).

Amino acids and vitamins are capable of accelerating physiological processes, and nutrients uptake. The observed difference in proline and sugar contents under saline sodic field or saline condition in pots may be attributed to the better salt tolerance and osmotic adoptability of putrescine-treated plants. Proline accumulation is a sign of salt tolerance, for its pivotal role in protecting intracellular macro-molecules and scavenging of hydroxyl radicals.

The observed increase in endogenous level of putrescine of treated plants insinuates the better response of wheat to exogenously applied putrescine, and these findings are in agreement with previous results. It is proven fact that polyamines have potential to be absorbed at different parts or surfaces of plants and thereby transported to xylem. Similarly, increased endogenous putrescine was observed in putrescine-coated leaves of apricot. The combined response of exogenously applied putrescine along with existing reservoir assists plants to cope against abiotic stresses.

The observed increase in antioxidant (SOD and POD) activities may be attributed to the positive role of putrescine in detoxification of reactive oxygen species (ROS) under salinity stress. The putrescine along with antioxidants acts as a defense mechanism against ROS. Putrescine and other polyamines scavenge free radicals within the nucleus and enhanced survival efficiency of cells.

Increase in endogenous phytohormones (IAA, GA, and ABA) in response to putrescine foliar application might be attributed to the enhancement of the phytohormones precursors.
General Introduction

Putrescine is also associated with degradation of enzymes that inhibit phytohormones. The polyamines have the potential of conjugates formation with phenols; thereby increase IAA biosynthesis. Previous results also demonstrated the increase in IAA contents of putrescine treated Chicory (*Cichrium intybus* L.), (Bais and Ravishanker, 2001). Increase in IAA and GA contents is also in agreement. The increase in the phytohormones is correlated positively with plant height, fresh weight and could be attributed to increase cell division and incell proliferation. ABA is a stress responsive hormone which is associated with stomatal responses of plants. Exogenous application of polyamines including putrescine is associated with increased production of ABA.

Putrescine application increased the seed yield by 30% over control. The increase in yield parameters was more pronounced under un-stressed conditions than stressed. Under salt stress increase in cations (Na, K and Ca) leads to amino acid and protein s degradation and polyamines are involved with detoxification of these cations. The decrease in the affectivity of polyamines effectiveness under salt stress is correlated with osmotic signals for their initiation. The role of exogenously applied polyamines in flowering, pollination, and yield improvement of Kiwi fruit (*Actinidia deliciosa* (A.Chev.) C.F. Liang & A.R. Ferguson) and cotton (*Gossypium barbadense* (L) plants has been demonstrated previously.

5.4. Conclusion

The application of putrescine, may have a positive influence on the selectivity of ions in the process of absorption, transport metabolism, and regulation of Na in salt-stressed plants, grown in pots or saline-sodic field. The accumulation and translocation of K, Ca, and Mg in stressed wheat leaves might be a key factor of alleviating the deleterious effects of salts. The improved elemental contents in leaves following putrescine application helped the plants to improve growth. The putrescine induced salt tolerance was mediated by the enhanced
production of sugar, proline, stimulation in the activities of antioxidant enzymes Superoxide dismutase and peroxidase. Furthermore, the improved endogenous putrescine positively modulated the phytohormones contents, thereby resulting increased biomass and yield of wheat under salt-stressed or unstressed conditions. Though the stimulatory effects of putrescine on wheat were less than tryptophan addition with PGPR and carrier based biofertilizer but putrescine application had been proved better than single inoculation of PGPR specifically for physiological parameters. In future, combined effects of PGPR used in this study (*Pseudomonas moraviensis* and *Bacillus cereus*) may help to understand the interaction of polyamines with microorganism and beneficial effects on crops. Putrescine application to plants enhances the endogenous putrescine. The increase in yield attributes and benefit cost ratio of putrescine treated plants reveled the polyamine putrescine application as a economically feasible way to combat salinity stress. Its effect is also significant under natural condition in the field.

7. Concluding chapter

Both the strains (*Pseudomonas moraviensis* and *Bacillus cereus*) were phosphate solublizer but *Pseudomonas moraviensis* had greater potential than *Bacillus cereus*. Both the PGPR showed inhibition against pathogens *Helminthosporium sativum* and *Fusarium moniliforme*. The survival efficiency of PGPR measured as colony forming unit revealed greater tolerance of both the microbes in the presence of rhizospheric soil filtrate containing mixture of salts (Na$_2$CO$_3$,NaHCO$_3$ and NaCl, EC: 4.5dSm$^{-1}$) as compared to that of NaCl alone. *Bacillus cereus* was less tolerant to salts mixture (NaCl, Na$_2$CO$_3$ and NaHCO$_3$) present in the saline soil of the field than *Pseudomonas moraviensis*. However, both the PGPR exhibited similar magnitude of tolerance to NaCl. Both the PGPR had the potential to synthesize IAA but
**Bacillus cereus** produced more IAA in the culture as compared to that of *Pseudomonas moraviensis*.

Colony forming Unit (cfu) of both *Pseudomonas moraviensis* and *Bacillus cereus* increased linearly with incubation period (40 d) in the presence of carrier material used in the biofertilizer. The coinoculation of *B. cereus* and *P. moraviensis* in carriers showed greater cfu over that of single inoculation in the same carrier. Decreases in Na and Cl, the Na⁺/K⁺ and Na⁺/Ca⁺ of soil and leaves were more pronounced when PGPR were coinoculated with carriers. Percentage increase in cfu and the effects of PGPR on physiological parameter of both PGPR was higher in sugarcane husk. The commercialization and application of biofertilizer on large scale and on different crops might be helpful for sustainable agriculture under saline condition. The greater cfu is the indicative of carrier induced potential of the PGPR and assisted in proliferation because of rich C-source and better moisture holding capability of sugarcane husk for the growth of PGPR bioinoculants.

Addition of tryptophan to the culture media increased the growth of PGPR (Fig 3.3). The application of tryptophan alone in the soil showed no significant changes on nutrients status, growth, physiology and yield parameters as compared to control but in combination with PGPR, these effects were many folds higher than control. Addition of tryptophan with PGPR ameliorated the deleterious effects of salinity by decreasing EC and SAR of soil which was most effective over carrier based biofertilizer and halophyte root powder treatment. *Bacillus cereus* addition with tryptophan was more beneficial than *Pseudomonas moraviensis* due to better tryptophan conversion and resulted in improved growth, proline, antioxidant enzymes and phytohormone contents of treated leaves. It is suggested that the synergistic behaviour of PGPR to added tryptophan, higher accumulation of proline and antioxidant enzymes in treated plant can ameliorate the deleterious effect of salt stress.
Halophyte root powder treatment improved the nutrient availability and organic matter thereby increased the proline, antioxidant enzymes and phytohormone contents (IAA, GA and ABA). Halophyte root powder may be applied as carrier with the consortium of PGPR for the formulation of biofertilizer. The need is to re-inoculate the root powder (used as carrier) with the PGPR residing there, in order to get synergistic action and better performance as biofertilizer.

PGPR induced decline in EC and SAR of rhizosphere soil of saline sodic field and potted plants grown under stressed condition, and magnitude of decrease was more pronounced in potted plants. Greater decreases in EC and SAR are perhaps, due to the sole effects of PGPR in sterilized soil whereas in field interactive effects of climatic and edaphic factors modulate the effects and also there is a competition of the PGPR indigenous microflora.

The magnitude of increases in proline, antioxidant enzymes and phytohormone contents were higher in field grown plants when treated with PGPR and PGPR + tryptophan because the interaction effects of climate with PGPR and plants produced more ROS. Hence, the tolerance strategy may be adapted to make antioxidant system more efficient to accumulate salt induce oxidative stress.

The application of carrier based biofertilizer may increase the farmer’s benefits by 24% and 42% in salt stress and unstressed condition respectively. L-tryptophan addition may increase the farmer’s benefits by 12% and 34% in salt stressed and unstressed conditions respectively. The halophyte root powder application on wheat may increase the farmer’s benefits by 33% and 39% in salt stressed and unstressed condition respectively. Putrescine foliar application was fewer beneficiaries for farmers with 8% and 16% farmer’s benefits under salt stress and unstressed condition respectively.
Though the stimulatory effects of putrescine on wheat were less than tryptophan addition with PGPR and carrier based biofertilizer but putrescine application had been proved better than single inoculation of PGPR specifically for proline and antioxidant enzymes. In future, combined effects of PGPR used in this study (*Pseudomonas moraviensis* and *Bacillus cereus*) may help to understand the interaction of putrescine with microorganism and their beneficial effects on crops.

The ABA contents of leaves of inoculated plants were higher than that of IAA. Among treated plants, highest IAA and ABA contents in soil and leaves were observed when PGPR were applied with tryptophan. The combined role of IAA and ABA due to PGPR application may act as an adoptive mechanism to cope the effects of salinity.

The coinoculation of PGPR resulted higher ABA in leaves than that of root powder treatments. PGPR inoculation alone and with different growth substances was more effective under stressed condition, however, the effects of PGPR under unstressed condition were higher for nutrient accumulation and yield attributes.

The mutant application on wheat in axenic condition showed less seed germination and growth than wild strain. Further screening of auxotroph mutants on molecular level is necessary to understand the role of IAA using IAA deficient mutants.