EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) AND FARMYARD MANURE (FYM) AMENDMENT ON GROWTH PARAMETERS AND ANTIOXIDANT LEVEL IN PADDY (ORYZA SATIVA L.) CROP UNDER SOIL SALINITY

Thesis

SUBMITTED TO
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW

FOR THE DEGREE OF
Doctor of Philosophy
IN
ENVIRONMENTAL MICROBIOLOGY

Submitted By
Shobhit Raj Vimal
(Enrolment No- 223/10)

Under the Supervision of
Dr. Jay Shankar Singh

DEPARTMENT OF ENVIRONMENTAL MICROBIOLOGY
(SCHOOL FOR ENVIRONMENTAL SCIENCES)
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University, NAAC Accreditation 'A' Grade)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025
UTTAR PRADESH, INDIA
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DECLARATION

I, **Shobhit Raj Vimal**, hereby declare that this Doctoral Research Work Thesis entitled "**Effect of plant growth promoting rhizobacteria (PGPR) and farmyard manure (FYM) amendment on plant growth parameters and antioxidant level in paddy (*Oryza sativa* L.) crop under soil salinity**" is my own work carried out under the guidance of **Dr. Jay Shankar Singh,** Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, (A Central University), Lucknow-226025, Uttar Pradesh, India. The matter embodied in this Thesis is written by me and has not been submitted to any other university for award of any other Degree or Diploma. This Thesis is essentially free from all kinds of plagiarism.

Date: 06/09/2018

Place: BBAU-Lucknow

Shobhit Raj Vimal

(Shobhit Raj Vimal)

CERTIFICATE

This is to certify that the Thesis entitled "**Effect of plant growth promoting rhizobacteria (PGPR) and farmyard manure (FYM) amendment on growth parameters and antioxidant level in paddy (*Oryza sativa* L.) crop under soil salinity**" submitted by **Mr. Shobhit Raj Vimal** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The Thesis submitted to Babasaheb Bhimrao Ambedkar University satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2008/2010/2013* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 06/09/2018

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Shobhit Raj Vimal

(Shobhit Raj Vimal)

ABBREVAITATION

ABBREVIATIONS

ANOVA	:	Analysis for Variance
AR	:	Analytical Reagent
BLAST	:	Basic Local Alignment Search Tool
bp	:	Base Pair
BSA	:	Bovine Serum Albumin
$CaCO_3$	:	Calcium Carbonate
CAS	:	Chrome Azurol S
CMC	:	Carboxy Methyl Cellulose
DAS	:	Days after Sowing
DMSO	:	Dimethyl Sulfoxide
DNA	:	Deoxyribonucleic Acid
dNTP	:	Deoxynucleotide Triphosphate
EC	:	Electrical Conductivity (dS m^{-1})
ECe	:	Soil Saturation Extracts EC (dS m^{-1})
g	:	Gram
g L^{-1}	:	Gram per Litre
h	:	Hour
HCN	:	Hydrogen Cyanide
$HgCl_2$	:	Mercuric Chloride
IAA	:	Indole-3-Acetic Acid
MEGA	:	Molecular Evolutionary Genetics Analysis
$MgCl_2$	:	Magnesium Chloride
mL	:	Millilitre

mM	:	Millimolar
N	:	Number of Observation
NA	:	Nutrient Agar
NaCl	:	Sodium Chloride
NCBI	:	National Centre for Biotechnology Information
ng/µL	:	Nano-gram per Microliter
NJ	:	Neighbor Joining
No.	:	Number
NS	:	Not Sufficient
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
PGP	:	Plant Growth Promotory
pH	:	Potential of Hydrogen
R	:	Correlation Coefficient
r-DNA	:	Ribosomal DNA
r-RNA	:	Ribosomal Ribonucleic Acid
SDW	:	Sterile Distilled Water
SEM	:	Scanning Electron Microscopy
SLR	:	Simple Linear Regression
SPSS	:	Statistical Package for Social Sciences
TAE	:	Tris-Acetic acid-EDTA
UV	:	Ultra-violet
$\mu g\ mg^{-1}$	:	Microgram per milligram
°C	:	Degree Celsius

CONTENT

CONTENTS

Different aspects of Plant Growth Promoting Rhizobacteria In Plant Growth Promotion

III. LOCATION OF SOIL SAMPLING SITES, ISOLATION AND CHARACTERIZATION OF SALT TOLERANT PLANT GROWTH PROMOTING RHIZOBACTERIA 34-66

Materials and Methods

Material and Methods
Soil Sampling and analysis of Relevant Physicochemical Properties

Material and Methods
Paddy Plant Growth Parameters and Rice Grain Yield

Results

CHAPTER 1

INTRODUCTION

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INTRODUCTION

Today, the demand for food is on rise and unexpected population growth critically slammed food security, arms an worrying moment for regulation of sustainable agro-food systems. By 2050, Global food demand is expected to raise 70-110 % for figuring out Global hunger (Laurance et al., 2014). With two billion more mouths to nourish by 2050, even brief failure in today's food productivity and supply structures, could easily led to serious starvation and even civil unrest, especially in developing countries (Wheeler and Braun, 2013). The contents of a typical food basket anywhere in the world today are limited to a small number of crops, with merely 20 plant species comprising 90% of the world's calories. About 75% of crop variety has been missing from farmer's field during last millennia, and forecast seems that more than 20% of the wild relatives of the some essential food crops will appear reduced from land by 2055, due to climate transformation (FAO, 2010). Deforestation, urban expansions, farming and other human activities have substantially changed Earth panorama. The per capita consumption growth of population inhabitants was standing responsible for increasing pressure on atmosphere. Comprehensive studies of worldwide food styles revealed that human diet plan globally have expanded 36% more similar in structure over the previous five decades (Khoury et al., 2014).

The expected rise in temperature and decreased precipitation owing to climate change and unabated anthropogenic activities add complexity and uncertainty to agro-industry. The impact of soil nutrient imbalance, mismanaged use of chemicals, high temperature, flood or drought, soil salinity, and heavy metal pollutions, with regard to food security, is increasingly being explored worldwide. Soil salinity is major abiotic stresses affected plants very badly. The global extend of primary salt-affected soils is about 955 Mha, while secondary salinization affects about 77 Mha, with 58% of these in irrigated areas (Tajgardan et al., 2010). Nearly 20% of all irrigated land is salt-affected and this proportion tends to increase in spite of considerable efforts dedicated to prevent land degradation and to secure sustainable land use and management (Siddikee et al., 2010). The most affected soils are situated in Hungary, Romania, Greece, Italy and the Iberian Peninsula (Silva and Fay, 2012). According to global change scenarios, rising sea level will threaten agricultural production in large areas of hitherto fertile lands by increasing the salinity of the soil (Jha at al., 2011). Salinization consists of an accumulation of water soluble salts in the soil. These salts include the ions potassium (K^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), chloride (Cl^-), sulphate (SO_4^{2-}), carbonate (CO_3^{2-}), bicarbonate (HCO_3^-) and sodium (Na^+). Sodium accumulation is also called sodification (Silva and Fay, 2012). These concentrations of soluble salts through their high osmotic potential affects plant growth by restricting the uptake of water and balanced absorption of essential nutritional ions by the roots (Tester and Davenport, 2003).

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination density, plant vigour and crop yield, limiting nutrient absorption and reducing the quality of the available water. For example, elevated salinity weakens plants due to the increase in osmotic pressure and the toxic effect of the salts (Munns and Tester, 2008; Paul and Lade, 2014). Saline soils show the following physical-hydric characteristics: low permeability, low hydraulic conductivity and aggregate instability (Freire, 2009). Salinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency (Netondo et al., 2004; Barnawal et al., 2017). Salt stress as one of the most widespread abiotic constraints in food production may also result in the negative ecological, social and or economic outcomes. Agricultural crops drastically affected in high salt concentration. High salt concentration lower down crop production and affect soil physiochemical and ecological balance of the ecosystem. Successful remediation of salt degraded areas for crop production, based on sustainable management practices evolving efficient, low cost, easily adaptable methods, is the challenge.

Rice (*Oryza sativa* L.) is a vital agent for nutritional security and according to report of International Rice Research Institute (IRRI)-Philippines, 90% of global production of rice is consumed by the Asian population. Food and Agriculture Organisation (FAO)-United Nations, data shows (*http://faostat3.fao.org*), after sugar cane the next three first crops in terms of production (million tonnes) in the world are the cereal maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). In 2011, global production of rice was almost 723 MT. Seeds utilizes different prompts from the earth to terminate dormancy and where and when to set up another plant. Primary signals utilized by seeds for sprout as part of world compromise light, nutrients, suitable heats, water substance, and likely different signs. Undisputedly plants are flexible in environment with the possibility to build up a plenty of morphological patterns relying on growth conditions to which they are exposed. This morphological flexibility has empowered plants to colonize almost every edge of globe and to survive in the harshest conditions. Rice exhibits wide adaptability to different environments, which makes it the most widespread crop in the world. It can grow in saline condition, drought conditions or in shallow water (up to 50 cm of water), and in a wide range of latitudes and up to 3000 m altitude. For this reason, it is considered a strategic crop for food security in the world by the FAO. However methodologies have been utilized aiming at enhancing multiple stress resilience in early decades but inadmissible results occurs. Escalating salinity fields imposed severe osmotic damages on paddy plant and declined grain productivity. Applications of agrochemicals in rejuvenation of saline soils drastically deteriorate soil physicochemical and biological properties. Thus, it is now necessary to these global concerns somewhat mitigated through upgraded sustainable administration.

The emerging environmental issues such as soil pollution, land degradation, loss of soil fertility or soil microbial diversity, or rise of average global temperatures, among many others, are adversely impacting the various ecosystems (Singh and Gupta, 2018). The changes in plant and animal communities are relatively slow, but alterations in soil microbial community compositions and their ecological functioning happen much more rapidly, but mostly stay ignored. The soil microbial communities are the key responders to any environmental change, but details on what exactly happens to microbial community composition and their functional role as a response to variations in soil parameters changes to most of the cases are still quite unclear. Microbes, a tiny living with unmatchable capacity offer an innovative and feasible option in agriculture and got position of decent bioengineers for engineered tainted agro-ecosystems (Singh, 2015). Beneficial microbes associated with plants rhizosphere are known to stimulate plant growth and enhance plant resistance to biotic (diseases) and abiotic (salinity, drought, pollutions, etc.) stresses. Roots are the vital part of plant can taste which supplements are available in soil and answer is easily shown in plant health. They can also taste and integrate signalling by means of different chemicals substances that are delivered in their distinctive organs, as well as by microorganisms, plants, and animals in their environments (Cheynier et al., 2013; Fonseca et al., 2014). Root exudate varies with plant genotypes, recruit microbial partners towards and along roots (Patel et al., 2015). Rhizosphere microbial counts go beyond to 1×10^{11} microbial cells per gram root (Egamberdieva et al., 2008) in eutrophic while it has decline up to 10^{4} under stressed terrestrial ecosystems. Microbes under rhizosphere can trustily modulate root exudate patterns (Patel et al., 2015), improves rhizospheric architecture (Vimal et al., 2017) and induce systemic resistance to consequent pathogen attack (Glick, 2014).

Plant-growth-promoting-rhizobacteria (PGPR), a key component of soil micro-biota, could play vital roles in the maintenance of plant fitness and soil health under stressed environments. The PGPR got special promotions among soil microbes during last few decades due to unmatchable capability. After successful tuning with the plant roots rhizobacteria utilizes carbohydrates, amino acids, organic acids and exercises with different plant growth promontory traits (Choudhury et al., 2014). PGPR boost plant vigor by means of different mechanisms, altering root architecture (Grobelak et al., 2015), initiate phytohormone levels (Glick, 2012), pathogen reduction (Singh et al., 2013), stress tolerance (Vimal et al., 2018a) and comprehension these unpredictable cross-kingdom interactions inspires us into root formative science and bacterial signaling (Singh et al., 2011; 2015; 2018a, b). The PGPRs are proficient in modulating the root system architecture which is a critical factor of productivity (Singh, 2015). The capability of PGPR to influence plant development and root processes was excellently addressed by Hatesami and Maheshwari, 2018. By contrast, the mechanisms by which PGPRs up-regulate cell division, and improve the equilibrium between proliferation and differentiation in the primary root and lateral root initiation sites, remain largely unidentified (Veloccia et al., 2016). These adjustments are built up by changing plant endogenous signalling pathways. The PGPR got tremendous attention in to taking care of soil and plant health due to environmental calamities (Vimal et al., 2016; 2017; 2018a, b). The PGPR provides great promise in sustainable future soil fertility and crop productivity managements. The PGPR communications with their host plant have knocked the minds of researcher for advancement in PGPRs based microbial technology.

Manure, compost, biochars and other partially composed organic substances presume dynamic roles in fertility restoration in agricultural fields (Cavagnaro, 2014). Application of organic manures in stressed soils significantly enhances soil microbial activities, which improves root network and enhances plant architecture. Acidic nature of farmyard manure potentially inhibits severe harmful nematodes, pests and reduces disease severity. However manures amendment significantly regenerate soils physico-chemical properties. Furthermore, intensive animal husbandry practices have led to production of animal dung, which are the good sources of organic matter and nutrients as nitrogen and phosphorus. Mislead management of animal excreta results in different ecological contaminations. Natural and safe management through application in crop field are the easy solutions. Different soil processes as nitrogen fixation, nitrification, and de-nitrification, PO_4 solubilization, siderophore productions significantly correlated with microbial community structure of the soil (Bai et al., 2017). Countless number of microbes as algae, mycorrhizal fungi, plant growth promoting rhizobacteria (PGPR) processes in different nutrient cycling. Thus amendments of organic manures in stressed soils as bio-fertilizer, compost, and vermi-composts offers a blameless bio-agent in rejuvenation of degraded ecosystem.

The application of organic manure as a soil conditioner in combination with suitable salt tolerant PGPR strains could improve the soil-plant-microbe-interaction and may enhance the crop yield under stressed soil conditions. The PGPR in association with organic amendments like FYM may significantly reduce the amount of energy demanding inputs, such as chemical fertilizers and can contribute to mitigation and adaptation for paddy plant under saline soils. Investigations are needed in this area that how and how much of the PGPR with FYM amendments facilitates plant growth, enhanced paddy crop production and regenerate soil fertility. Thus, the present doctoral research hypothesizes that the addition of salt tolerant PGPR inoculant + FYM (as organic amendment) to paddy soil could be a novel and cost-effective tool to enhance soil fertility and alleviate the salinity stresses of paddy crop plants. The present study was conducted to investigate the efficacy of salt tolerant PGPR strain + FYM on paddy plant growth productivity with following 3 objectives.

(i) To analyze the impact of PGPR and farmyard manure (FYM) applications on soil physico-chemical properties.

(ii) To examine the impact of PGPR and FYM applications on plant growth parameters and antioxidant level in paddy crop in saline soil.

(iii) To assess the correlation between the PGPR and FYM application and plant growth parameters and yields.

Note: For more clarity it is important to mention here that whole experimental work of present study broadly may be divided into two parts.

Part I	**Part II (3 objectives)**
Isolation and characterization of salt tolerant plant growth promoting rhizobacteria from saline soils (Chapter 3)	Application of isolated salt tolerant most efficient PGPR strain *C. albidum* SRV4 in combination with FYM in field conditions (Chapters 4, 5, 6 and 7)

CHAPTER 2

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

Soils are a finite natural resource and are non-renewable on a human time scale. The primary challenge till date in agricultural sciences is to introduce an innovative technology that easily accessible for grass root farmers. The agrochemical fertilizers are easily available in market and farmers are applying these harmful chemical to the crop fields without hesitation. That’s the basic problem for land fertility beyond intensifying degraded ecosystem. Since soils are the foundations for food, animal feed, fuel and natural fiber production besides the range of ecosystem functioning thus, generating awareness on the life-supporting functions of soil become necessary for production. Even abnormal and atypical changes in weather pattern works as a role model for crop deterioration and yield losses. The human population, which doubled in the last forty years, is expected to double again within the coming half century. By 2050 the world population will expected to cross 9 billion, with provide nutritional security for hungry stomach are major global challenge (FAO, 2013). The FAO explained nutritional security as “Physical and economic accesses to sufficient, hygienic and nutritional food for an active and healthy living”. Therefore, there is need to develop eco-friendly technologies that can be viable and sustainable for enhancing the crop productivity for coming generation without damaging the ecosystem and environment.

2.1 Environmental Stresses and Plant

Plants, a flexible organism on planet, could grow on every corner and survive in the harshest environments. Plethora of morphological configurations supports plants to live under versatile conditions. However, the standing and aquatic plant got enormously affected by different abiotic and biotic stresses which not only disturbs plant health but deteriorate different vegetative flora around the Globe (Laurance et al., 2014). The abiotic conditions such as nutrients, water, light, temperature, gasses, and magnetic fields are invariably diminish plant health depending on intensity (Choudhury et al., 2014).

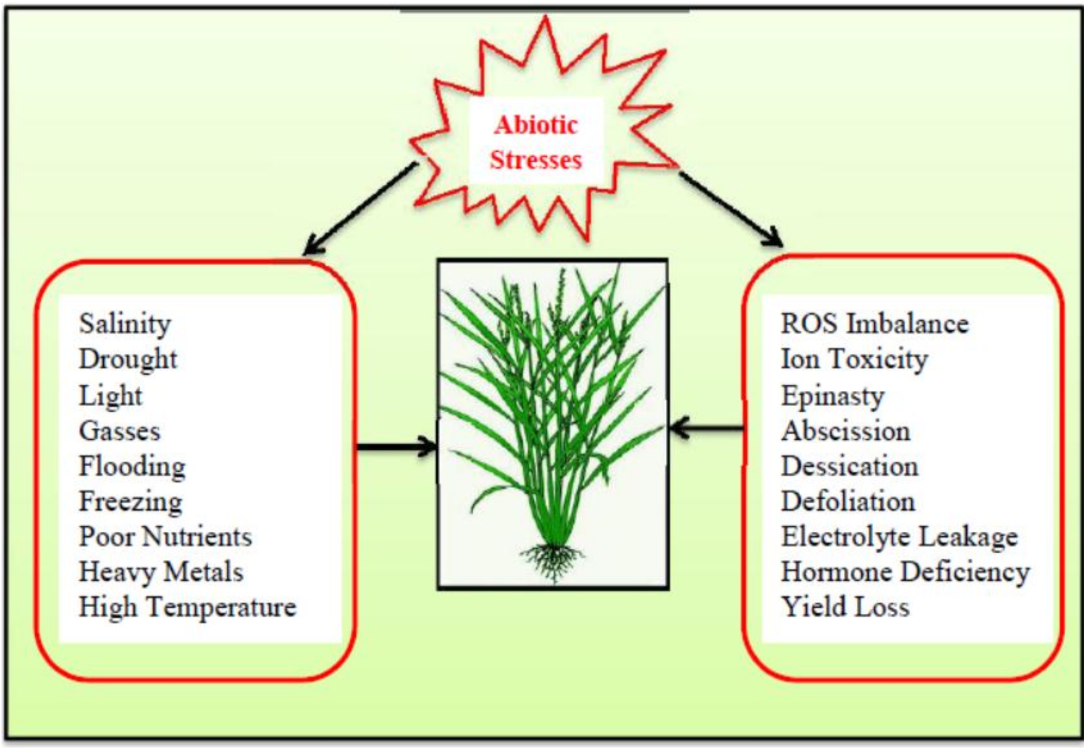


Figure 2.1 Effect of different abiotic stresses on paddy plant

Under proper extent they all are motivated plant health but their shifting attention desperately interrupt plant vigour. The Indian subcontinent will be heated by 0.57°C in the last 100 years and models depicted that it is likely to further rise 2.5°C by 2050 and 5.8°C by 2100 (Kumar et al., 2006; Grover et al., 2011). The irrigation requirement in arid and semi-arid regions is estimated to enhance by 10% with every 1°C rise in global temperature. Abiotic factors displays serious economic losses are likely to result as a result of global warming. The biotic stresses like pathogens, insects, nematodes, etc. directly feed and getting nutrition from the plant. Large population of these biotic components utilizes plant as their energy source and consume the whole field crops in various areas.

2.2 Soil Salinity Problems

Soil salinity is a major environmental abiotic constraint affecting more than 100 countries, 900 million hectares of land, accounting for nearly 6% of the world's total land surfaces worldwide (Paul and Lade, 2014). The United Nations Environment Program (UNEP) estimates that approximately 20% of agricultural lands and 50% of crop lands got affected with excess salt concentrations globally (Jamil et al., 2011). When salts are more soluble than $CaCO_3$ and $CaSO_4.2H_2O$ are present in the soil and deteriorate crop health and grain productivity of most crops these soils are considered as salt affected. Saline soils featured as electrical conductivity of the saturation soil extract of more than 4 dS m^{-1} at 25 °C (Richards, 1954). Salinized fields continuously raised by 10% annually due to severe anthropogenic sources as well as natural calamities as low precipitation, surface evaporation, irrigation with high salt water and poor agricultural practices.

Table 2.1 Response of plants on different range of soil electrical conductivity

Soil electrical conductivity (ds m^{-1})	**Soil characteristics**	**Plant responses**
0-2	Normal soil	Mostly negligible
2-4	Normal soil	Growth of sensitive plants is restricted
4-8	Slightly saline soil	Growth of many plants is restricted
8-16	Moderately saline soil	Only tolerant plant grows satisfactorily
Above 16	Severely saline	Only a few, very tolerant plants can grow

Soluble salts most commonly present in soils are chlorides and sulphates of Na^{+}, Ca^{++} and Mg^{++} (Grover et al., 2011). The predominant salt is normally NaCl "table salt". Saline soils are therefore also sodic soils but there may be sodic soils that are not saline, but alkaline. Salt export matches salt import and salt will not accumulate and causes salinity (Choudhury, 2012). Salinity may also affect other soils to a lesser extent and may lead to recognition of saline phases which also deserve attention when present in salt sensitive crops. Unproductive/Low productive agro-ecosystems directly affected economical as well as diminish societal wealth.

2.3 Paddy Crop Productivity

Paddy crop is grown in more than hundred countries around world with a cumulative harvested part of 158 million hectares producing more than 700 million tons yearly (470 million tons of milled rice). Asia alone represents 90% of the Global paddy production with statics of 640 million ton. Sub-Saharan Africa produces about 19 million tons and Latin America some 25 million tons. In Asia and Sub-Saharan Africa almost all rice grown on small agricultural farms of 0.5-3 hectares (FAO, 2013).

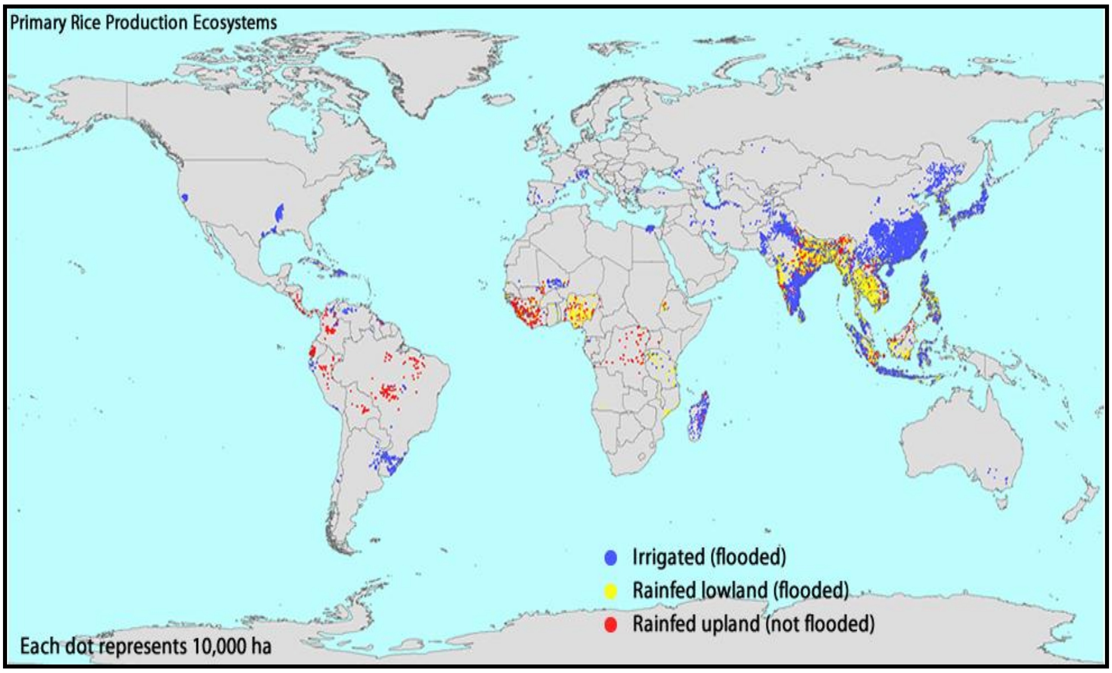
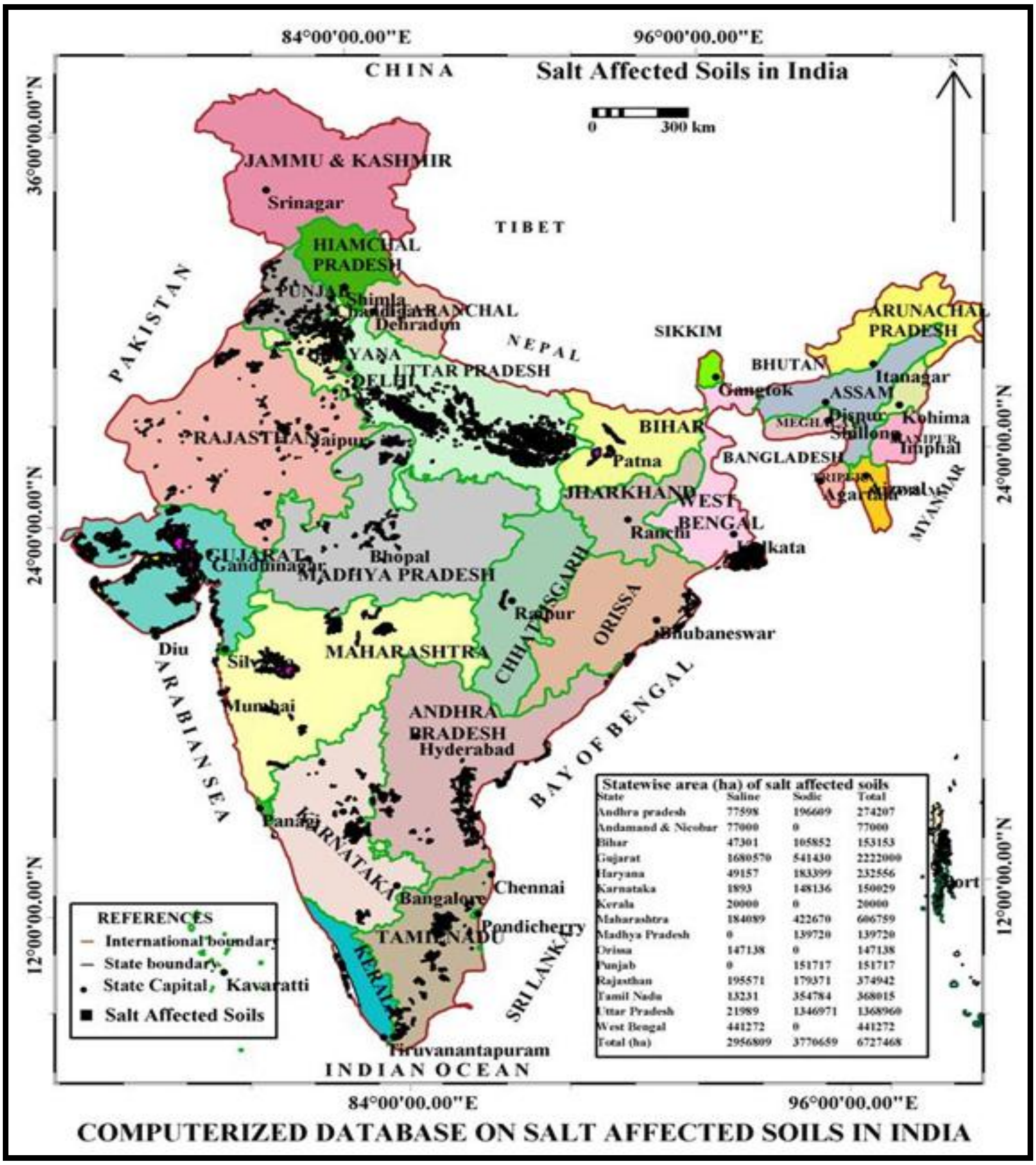


Figure 2.2 Global primary rice production ecosystems in world

The paddy productivity ranges from ≤1 tons ha^{-1} under very poor rain-fed conditions to more than 10 tons ha^{-1} in the well watered conditions. Rice is the staple food for three fourths of the Indian population. In the Global context, India stands first in area with 43.7 million hectares and second in production with 92.24 million metric tons (Chithrashree et al., 2011). Small and in many areas shrinking, farm sizes account for the low incomes of rice farm families. The paddy crop established themselves in different environments and become productive while other crops become fail to survive.

2.4 Soil Salinity and Paddy Agriculture

Soil salinization is Global environmental problem in irrigated fields tempered crop lands by 1-2% annually, hitting hardest in the arid and semiarid regions globally, resulting in poor and little crop production. Worldwide, the major factor in the development of saline soils is a lack of precipitation. The crop field irrigation is the primary anthropogenic source of salinization. River, canals or ground water practices in irrigation is potential sources of soil salinity. Evaporation leads to salts presence on applied field surfaces and affects plant health. Excess salts retard growth of crops and constraints production. In severe cases salinization causes land to be abandoned. In irrigated area where water management is poor the salt can be brought to the soil surfaces by capillary transport and form a rising water table. These salts inhibit roots growth and further evaporation lead to soils ruins. The excess salt ions are directly responsible for hyper-ionic and hyperosmotic conditions and distract nutrient absorption efficiency of roots and possess stressed environment for plants. High salinity is commonly caused by high concentrations of sodium (Na^+) and chloride (Cl^-) ions in the soluble fraction of the soils. Na^+ accumulation decays soil porosity, condense soil aeration and reduced water conductance (Porcel et al., 2012). Its tuff for plant to uptake nutrients from such stressed soils. In irrigated areas where salinity is stable, the salt concentration of the drainage water is normally 5 to 10 times higher than that of the irrigation water. The food and agriculture organization (FAO) of the United Nations (2010) predicted about 250 million hectares of irrigated land in the world, nearly 50% already show salinization and soil saturation difficulties, and 10 million hectares are abandoned yearly due to these problems.



Statewise area (ha) of salt affected soils

State	Saline	Sodic	Total
Andhra pradesh	77598	196609	274207
Andamand & Nicobar	77000	0	77000
Bihar	47301	105852	153153
Gujarat	1680570	541430	2222000
Haryana	49157	183399	232556
Karnataka	1893	148136	150029
Kerala	20000	0	20000
Maharashtra	184089	422670	606759
Madhya Pradesh	0	139720	139720
Orissa	147138	0	147138
Punjab	0	151717	151717
Rajasthan	195571	179371	374942
Tamil Nadu	13231	354784	368015
Uttar Pradesh	21989	1346971	1368960
West Bengal	441272	0	441272
Total (ha)	2956809	3770659	6727468

Figure 2.3 Salt affected fields in India (Source CSSRI)

Rice is relatively susceptible to excessive concentration of salts (Howell, 2001), and currently, 30% of worldwide paddy fields are affected by excess salinity (Paul and Lade, 2014).

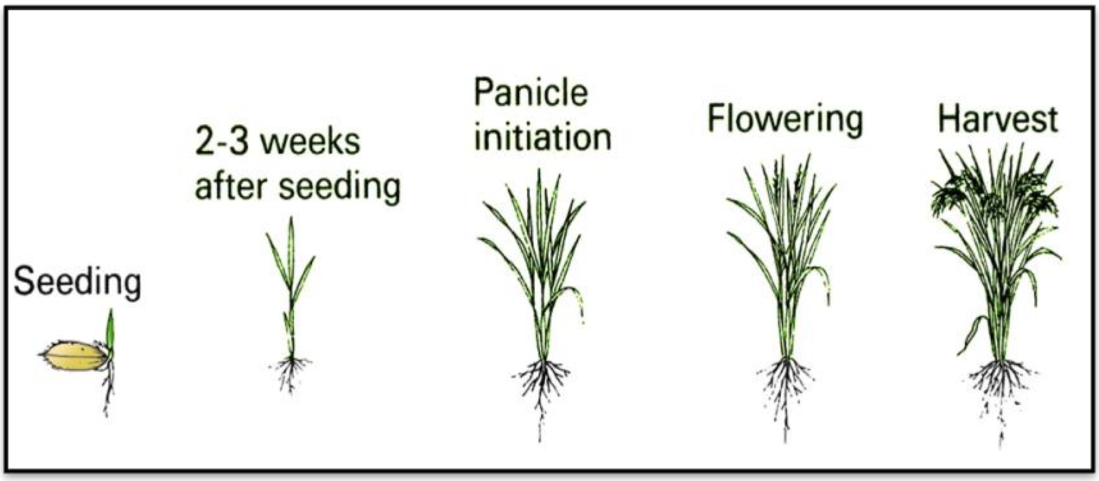


Figure 2.4 Life cycle (different growth stages) of paddy crop

The soil salinity diminishes a number of paddy plant growth parameters including seed germination, panicles initiation, tiller number, spikelet formation, delayed heading and rice grain size (Nakbanponte et al., 2014). Soil salinity causes alteration in Na^+ transport to shoot, accumulation of Na^+ in older leaves, high Cl^- uptake, low K^+, P and Zn uptake, decline in sucrose accumulation, derange nitrogen metabolism, higher portioning of dry matter to senescing leaves and reduction in energy content (calorific value) in dry matter. High salt level leads to imbalance of cellular ions, resulting in ion toxicity thus sponsored osmotic stress, and enhanced production of reactive oxygen species (ROS) (Grover et al., 2011). Na^+ and Cl^- are the major ions responsible for ion toxicity, causes adverse effects on plant growth and development (Paul and Lade, 2014). Excess Na^+ and more importantly Cl^- affect plant enzymes and causes cell swelling, reduced energy production and other physiological patterns (Nunkaew et al., 2015). The uptake and accumulation of Cl^- disrupt photosynthetic function through inhibiting nitrate reductase (NR) activity (Nadeem et al., 2014). Once the capacity of cells to store the salts is exhausted, their build-up in the intracellular space leads to cell dehydration and death (Kang et al., 2014).

During the onset of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy and lipid metabolism of paddy plants are affected (Paul and Lade, 2014; Hashem et al., 2015). Photosynthetic capacity of paddy plants has been found to considerably reduced due to the osmotic stresses and the partial closure of stomata (Glick, 2014; Barnawal et al., 2017). Paddy plants experiences membrane destabilization and endure nutrient imbalance (Nadeem et al., 2014; Vimal et al., 2018a).

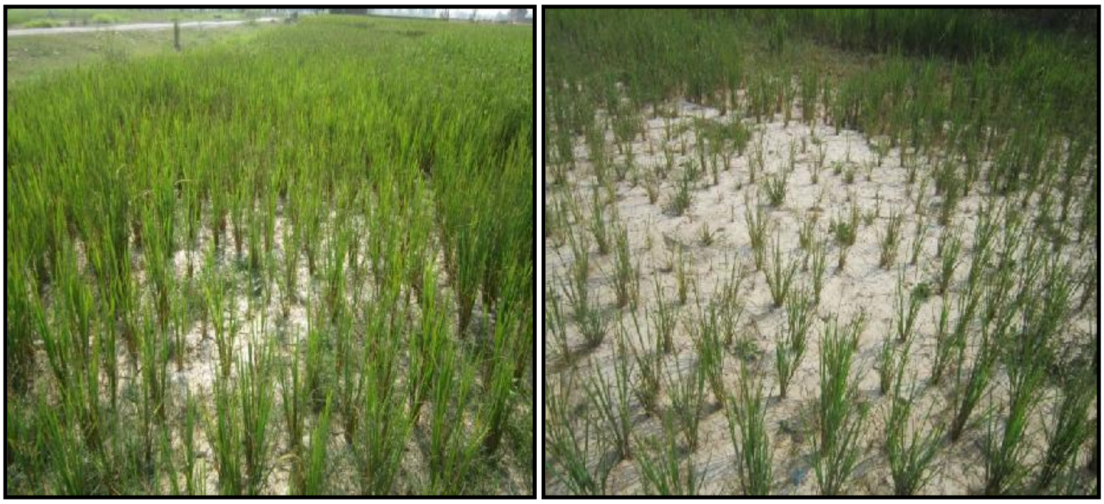


Figure 2.5 Excess salts appeared as white layers in a paddy crop field

Furthermore, plant responses to osmotic stress are reflected in terms of decreased cell growth and development, reduced leaf area chlorophyll content and accelerated defoliation and senescence (Kang et al., 2014). Thus, stress environments not only diminish crop yields but also deteriorate the soil fertility. Decline in production of major principle crops as paddy and continuous escalating food demands as a consequence of ongoing increase in population will be serious concerns for food security in near future. Ill-suited applications of agrochemicals for intensive crop production have undeniably posed negative and sometimes irreparable threats to the environment. Therefore, there has been a recent resurgence of interest in environmental friendly smart green revolution technology to enhance the crop yield.

2.5 Microorganisms in Saline Agriculture Soil

Agriculture which is based on agriculturally important microbial technology for replenishing agrochemicals will be an emerging and eco-safe technology for climate smart agriculture. Bioinoculants of agriculturally important microorganisms and deploy them on agricultural fields will provide cost effective and environmentally safe technology for farmers especially those who cannot afford expensive technologies. It has been submitted that typical one gram of soils contains about 9×10^7 bacteria, 4×10^6 actinomycetes, 2×10^5 fungi, 3×10^4 algae, 5×10^3 protozoa and 3×10^1 nematodes (Alexander, 1991; Glick, 2014). The role of microorganisms in improving nutrient availability to plants is an important factor (Pereg and McMillan, 2015; Hamilton et al., 2016). Beneficial interactions have been reported among plants and microorganisms in the stress environment and derived ecosystem productivity (Cosme and Wurst, 2013; Rashid et al., 2016). Application of microbes in combination with manures, improves the efficiency of applied fertilizers avaiablity to crop plants and ultimately the crop yields (Singh et al., 2011; Rashid et al., 2016). Microbial bio-fertilizer is ecologically feasible and economically suitable alternative of agro-chemicals for agricultures. Various investigations addressed for improving and understanding diversity, dynamics and importance of agriculturally important microbes and their beneficial and cooperative roles in crop productivity (Vimal et al., 2017). Microbial bio-fertilizer gains tremendous momentum for Global food security, enhancing crop productivity, sustainable rural livelihoods and land restoration. The PGPRs are proved as potential microbes for the growing necessity of sustainable agriculture with a holistic vision of environmental protection. The PGPRs have been successfully employed as bio-inoculant with other microbes in agriculture for enhancement of crop yields. PGPR Bio-fertilizers are ecologically feasible and economically suitable alternative of agrochemicals for farmers.

2.6 The Plant Rhizosphere

There is a thin layer of soil immediately surrounding plant roots that is an extremely important and active area for root activity and metabolism which is known as rhizosphere. The rhizosphere concept was first proposed by Lorenz Hiltner in 1904 to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities. It has been observed that bacterial cells even inside the rhizodermis of healthy roots. Huge number of bacteria, fungi, protozoa, and algae are similarly coexists in the rhizosphere. The rhizosphere micro-biome extends the functional repertoire of the plant beyond imagination. Plant genotype significantly recruits microbes for nutritional security (Patel et al., 2015). Root exudates exclusively provide food and attract microorganisms. This rhizosphere effect is caused by the fact that a substantial amount of the carbon fixed by the plant, 5-21%, is secreted, mainly as root exudate. This ample amount of root exudes attracts a number of microbes and enrich microbial communities. The concentration of bacteria in the rhizosphere is 10-1000 times greater than that in bulk soil; it is still 100-fold lower than that in the average laboratory medium. The PGPR naturally occurring soil bacteria that colonize plant roots and act as bio-fertilizer, bio-protectant, bio-stimulants and has gained worldwide importance and acceptance for agricultural benefits.

2.7 Plant Growth Promoting Rhizobacteria

Based on their experiments on *Raphanus raphanistrum* L., two eminent researchers Joseph W. Kloepper and M.N. Schroth from Department of Plant Pathology at University of California-Berkeley in 1978 introduced the term ‘rhizobacteria’ to the soil bacterial community that competitively colonized plant roots and enhanced plant growth. Kloepper and Schroth (1981) termed these beneficial rhizobacteria as plant growth-promoting rhizobacteria (PGPR). The PGPR can be defined as the indispensable part of rhizosphere biota that when grown in association with the host plants can stimulate the growth of the host. PGPR hold, a metabolically and functionally diverse group of soil-inhabiting bacteria, exhibit multiple mechanisms that suppress phyto-pathogens and promote crop growth. The PGPR facilitate the plant growth through diverse mechanisms, which include acquisition of resources (Bhattacharyya and Jha, 2012), enhance transformation and acquisition of nitrogen (N) (Bell et al., 2015), mineralization of organic phosphorus (P) (Bhattacharyya and Jha, 2012), production of phyto-hormones (Kurepin et al., 2015), exo-polysaccharide (EPS) production and synergism with other bacteria-plant interactions (Rashid et al., 2016). Stress mitigation potential of certain rhizobacteria strains are studied in detail and observe a significant positive result (Singh, 2015; Vimal et al., 2016). Under stress conditions, plants produce stress induced chemicals such as C_2H_4 that negatively affect plant growth. The PGPR have the ability to reduce the levels of stress-induced chemicals by producing enzymes such as the ACC deaminase and thus protect the plants against damages. The establishment of useful host defense enzyme sinks using PGPR community and the reduction in stress levels induce the elongation of roots, encourage the formation of branched roots, and minimize the hazardous effects of stress to promote the plant growth and viability.

Furthermore, rhizobacteria play crucial roles in plant-microbe interactions owing to their ability to produce phyto-hormones and promote phyto-stimulation efficiency. They can also protect plants through the control of soil and seed borne phyto-pathogens (Bach et al., 2016) and induction of systemic resistance (Jain et al., 2013), and the production of volatile compounds (Bhattacharyya et al., 2015) that can inhibit the growth of plant deleterious microbes. Novel PGPR strains having multifunctional genetic configurations could be a potent tool for plants to cope up with the harsh environmental conditions. Important aspects of PGPR are depicts in figure below.

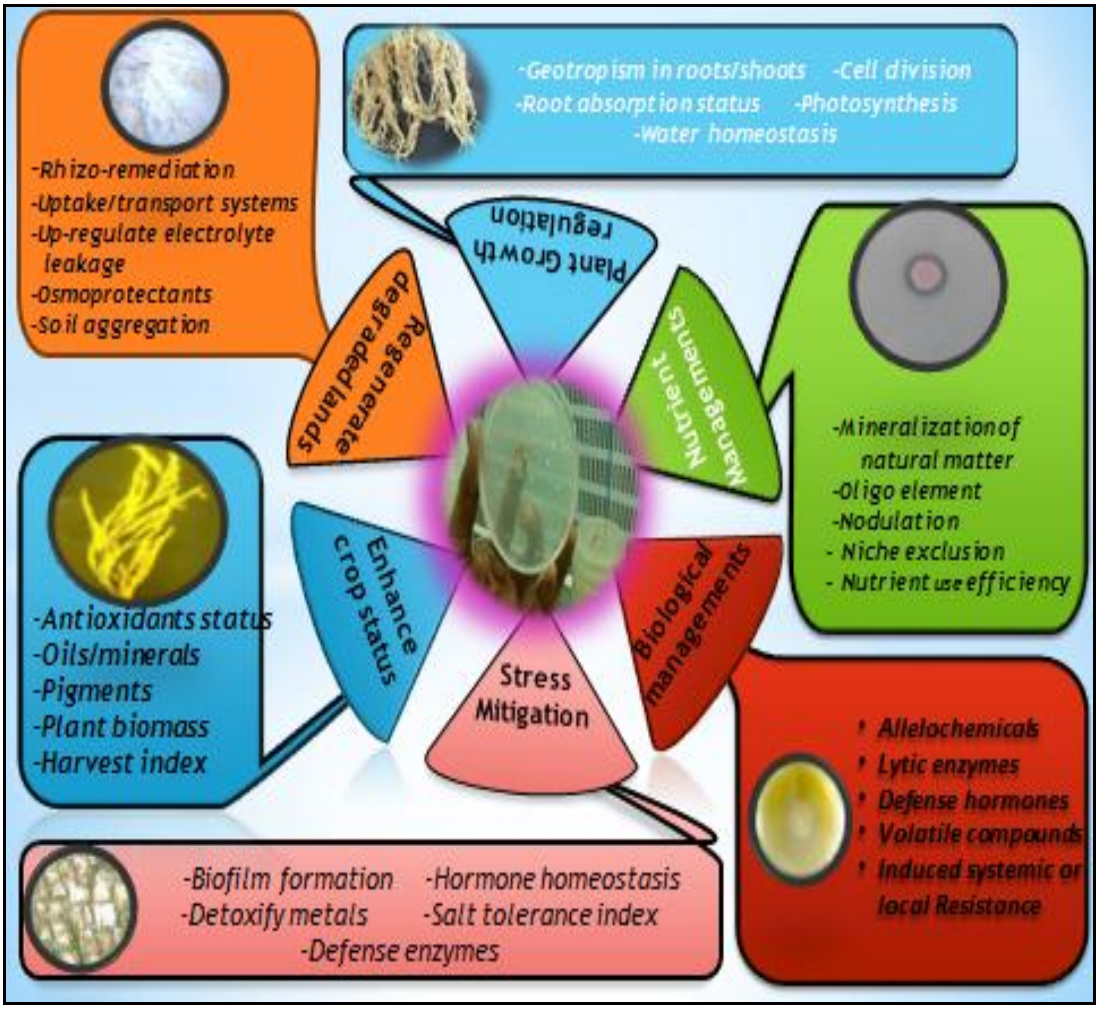


Figure 2.6 Different PGPR aspects in plant growth promotion

2.8 Different Aspects of Plant Growth Promoting Rhizobacteria in Plant Growth Promotion

2.8.1 Nitrogen (N_2) Fixation

Nitrogen is one of the key nutrients necessarily required for the improvement of growth and nutritional contents of plants (Krapp, 2015). N is reported to be the major nutrient required in sufficient amounts to sustain crop yield and quality (Sainju, 2013). Biological N_2 fixation (BNF) mediated by microbes contributes 180×10^6 t of fixed N per year globally, out of which 80% is contributed by symbiotic associations and the rest comes from free living or associative systems (Graham, 1988). Fixation of N_2 is a highly energy extensive process, requiring at least 16 mol of adenosine triphosphate (ATP) for each mole of elemental N_2 reduced, and it would be advantageous if the bacterial carbon (C) resources are directed toward oxidative phosphorylation which favours ATP synthesis, rather than glycogen synthesis, as the latter results in storage of energy as glycogen (Glick, 2012). In agricultural settings, perhaps 80% of the biologically fixed N comes from different PGPRs-*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium* of the family Rhizobiaceae in association with the leguminous plants. *Rhizobium* and *Bradyrhizobium* establish symbiotic associations with roots in leguminous plants such as soybean, pea, peanut, and alfalfa, convert N_2 into ammonia, and make it available to the plants as a source of N (Badawi et al., 2011). Symbiotic N_2 fixation is the well-known process exclusively driven by bacteria, the only organisms possessing the key enzyme nitrogenase, which specifically reduces N_2 to NH_3 in symbiotic root nodules (Zimmer et al., 2016). The *nif* genes (nitrogenase genes) are N_2 fixation genes and are present in both symbiotic and free-living systems (Kim and Rees, 1994). These genes include structural genes, genes involved in the activation of iron proteins, iron molybdenum cofactor biosynthesis, and electron donation, and regulatory genes required for the synthesis and function of enzymes. In diazotrophs, the *nif* genes are typically found in a cluster of around 20-24 kb with seven operons encoding 20 different proteins (Glick, 2012). The activation of *nif* genes in the symbiotic *Rhizobium* is dependent on low concentrations of oxygen, which in turn is regulated by another set of N_2 fixation genes, *fix* genes, which are common to both symbiotic and free-living N_2-fixing systems (Kim and Rees, 1994). The endophytes and *Frankia* species, both of which can symbiotically fix N_2 in association with the higher plants (Badawi et al., 2011). Thus PGPRs have efficient potentiality to reduce and minimize nitrogen fertilizer application. Supplementation of N_2 fixing PGPR as bio-fertilizer will sustainably rejuvenate degraded agriculture fields and enhance soils fertility.

2.8.2 Phosphate Solubilization

Phosphorus (P) is the second important key plant macronutrient for biological growth and development after nitrogen. Phosphorus is one of the most essential nutrient requirements in plants. An adequate supply of P is therefore required for proper functioning and various metabolisms of plants. Soils may have excessive phosphorous (P) but the amounts available to plants are usually a tiny proportion of this total. This low availability of phosphorous to plants is because of the vast majority of soil P is found in insoluble forms. Soluble P is often the limiting mineral nutrient for biomass production in natural ecosystems only taken up in monobasic ($H_2PO_4^-$) or dibasic (HPO_4^{2-}) soluble forms (Glass, 1989), and the elevated levels of heavy metals in soil interfere with P uptake and lead to plant growth retardation (Kudoyarova et al., 2017). Several phosphate solubilizing PGPRs reported to solubilize the insoluble form of phosphorus to soluble form through acidification, secretion of organic acids (Richardson et al., 2009) and chelation and exchange reactions (Hameeda et al., 2008). Sharma with other co-workers (2013) stated that assimilation of NH_4^+ within microbial cells is accompanied by the release of protons and solubilize phosphorus without production of any organic acids. Saprophytic bacteria and fungi are reported for the chelation mediated mechanisms (Whitelaw, 2000) to solubilize phosphate in soil. Small inorganic phosphorus dose applied to rhizosphere soils derive phytic acid mineralization by bacteria and results find improved plant phosphorus nutrition (Zhang et al., 2014). The ability of PGPRs to solubilize mineral phosphate, therefore, has been of immense interest to agricultural microbiologists since it can enhance the availability of phosphorus for effective plant growth. PGPRs have been recorded to solubilize precipitated phosphates to plants, representing a possible mechanism of plant growth promotion under field conditions (Verma et al., 2001). Of the various strategies adopted by microbes, the involvement of low molecular mass organic acids (OA) secreted by microorganisms has been a well-recognized and widely accepted theory as a principal means P-solubilization, and various studies have identified and quantified organic acids and defined their role in the solubilization process (Marra et al., 2012; Alori et al., 2017). Thus phosphorus solubilizing PGPR can be suitable bio-agent for microbial fertilizer and can be used for soil enrichment and smart agricultural practices.

2.8.3 Siderophore Production

Iron is an essential nutrient for almost all life forms. Despite being fourth most abundant elements in the earth crust and most types of soil occurs in excess (Radzki, 2013). The bioavailability of iron in many environments such as saline soil is limited by the very low solubility of the Fe^{3+} ion (Zuo and Zhang, 2011). In the aerobic environment, iron accumulates in common mineral phases such as iron oxides and hydroxides and becomes inaccessible to organisms. In most aerobic microbial habitats, Fe^{2+} is oxidized to Fe^{3+}, forming insoluble compounds that are unavailable to microorganisms. In these condition, few rhizobacteria produce low-molecular mass iron chelators with high affinity for iron termed as siderophores (Miethke and Marahiel, 2007; Machuca et al., 2007). These siderophores solubilize iron from minerals or organic compounds under limited iron conditions. Microbes (e.g. bacteria and fungi) have, therefore, evolved a strategy to acquire iron by releasing siderophores (Greek: “iron carrier”), small (generally less than 1,000 molecular weight) high-affinity iron-chelating compounds, which scavenge iron from the mineral phases by forming soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Broadly, siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron starvation (Miethke and Marahiel, 2007; Indira-gandhi et al., 2008). Siderophores, generally form 1:1 complexes with Fe^{3+}, which are then taken up by the cell membrane of bacteria, where the Fe^{3+} is reduced to Fe^{2+} and released from the siderophore into the cell (Boukhalfa and Crumbliss, 2002). Siderophore produced by majority of PGPR (Rajkumar et al., 2010) including rhizobia (Ahemad and Khan, 2012) has been suggested as one of the modes of growth promotion of nodulated legumes under field conditions where in siderophores facilitate the uptake of iron (assimilation) from the environment (Kloepper and Schroth, 1978; Katiyar and Goel, 2004). Siderophore producing plant growth promoting rhizobacteria can play tremendous role for microbial bio-preparations and become an suitable agent for agricultural production.

2.8.4 Exopolysaccharide (EPS) Production

In stress environments, plant health affected with proper nutritional imbalances. Under saline stress, excess sodium (Na^{+}) level distracts the absorption of other nutrients but also causes specific ion toxicity (Vimal et al., 2018a). Thus it is very essential to maintain Na^+/K^+ equilibrium to manage osmotic potential and stress adaptation in paddy plants. Certain PGPR strains show their ability to protect plants from the damaging effects of high Na^+ under saline soils. They do this by their ability of exo-polysaccharides (EPS) production. The EPS significantly reduced Na^+ uptake in the plant by binding it and biofilm formation (Qurashi and Sabri, 2012). The reduced availability of Na^+ results in lowering the uptake of Na^+ thereby maintaining high K^+/Na^+ content enables plant to survive better in salt stressed conditions (Khodair et al., 2008). The EPS also important in plants exposed under water deficit conditions. As drought conditions cause a negative influence on plants as well as on microbial population. EPS protect microbial flora as well as plants from desiccation, and permit them to continue their growth under water deficit conditions (Sandhya et al., 2009). Thus EPS producing PGPRs protects plant from different environmental calamities and restore soils properties.

2.8.5 Indole-3-Acetic Acid

Plant hormones are chemical messengers that affect a plant's ability to respond to its environment. Hormones are commonly organic compounds that are effective at very low concentration; they are usually synthesized in one part of the plant and are transported to another location. Microbial synthesis of the phytohormone auxin (indole-3-acetic acid/Indole acetic acid/IAA) has been known for a long time (Gordan and Weber, 1951). It is reported that 80% of microorganisms isolated from the rhizosphere of various crops possess the ability to synthesize and release auxins as secondary metabolites (Patten and Glick, 1996; Khan et al., 2016). Plant growth promoting rhizobacteria produces IAA interferes with many plant development processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Glick, 2012). Evidently, IAA also acts as a reciprocal signaling molecule affecting gene expression in several microorganisms. Generally, IAA affects plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light, gravity and florescence; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions. IAA produced by rhizobacteria likely, interfere the above physiological processes of plants by changing the plant auxin pool. Moreover, bacterial IAA increases root surface area and length, and thereby provides the plant greater access to soil nutrients. Also, rhizobacterial IAA loosens plant cell walls and as a result facilitates an increasing amount of root exudation that provides additional nutrients to support the growth of rhizosphere bacteria (Glick, 2012). Thus, rhizobacterial IAA is identified as an effecter molecule in plant microbe interactions, both in pathogenesis and phyto-stimulation (Spaepen and Vanderleyden, 2011). PGPR based bio-formulation shows their effectiveness in different plants health management and recovers from different stresses.

2.8.6 ACC Deaminase Production

Ethylene an important hormone endogenously produced by many plants is important in inducing various physiological changes in plants. Ethylene positively affects different aspect of plant health as root, stem laves, flowers, fruit development and ripening. Apart from being a plant growth regulator, ethylene has been established as a stress hormone (Saleem et al., 2007; Barnawal et al., 2017). Stress environments due to different abiotic and biotic factors significantly enhanced endogenous ethylene level and negatively affect plant growth (Glick, 2014). Elevated ethylene concentration induces defoliation and other cellular processes that may hamper plant growth and reduced crop performance (Bhattacharyya and Jha, 2012). Ethylene mediates wide range of plant responses at normal capacity ($\leq 10 \mu g\ L^{-1}$). At higher concentrations (≥25) ethylene may inhibit root, shoot elongation, suppress leaf expansion and stimulate epinasty (Glick, 2005; 2014). One-aminocyclopropane-1-carboxylic acid (ACC) is the direct precursor of ethylene synthesized by plants. Plant growth promoting rhizobacteria possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity can utilize ACC as a sole source nitrogen and deaminize ACC in to ammonia and α-ketobutyrate for use as carbon and nitrogen source. Thus declining ACC level positively correlated with stress induced ethylene concentration (Bharti et al., 2013; Barnawal et al., 2017). Under root zone a dynamic equilibrium of ACC concentration exists between root, rhizosphere, and rhizo-bacterium. Rhizo-bacteria utilize ACC as an energy source and successfully decline root ACC concentration and finally reduced root stress ethylene level. The ability of ACC-utilizing PGPR to ameliorate plant growth inhibition caused by stress induced ethylene through a decrease in ACC content (Glick, 2014) and ethylene production has been successfully validated (Barnawal et al., 2014). Wide range of PGPRs taxonomic group including *Pseudomonas, Achromobacter, Bacillus, Azospirillum, Agrobacterium, Acinetobacter, Enterobacter, Serratia* has been shown their effectiveness in stress mitigation through ACC deaminase activity (Nadeem et al., 2007; Cote et al., 2010; Wu et al., 2012; Ali et al., 2014). Thus potential PGPR strains possessing ACC deaminase activity may contribute to mitigating plant stress induced ethylene and promoting plant growth in salt stressed soils.

Table 2.2 Different Plant-Growth-Promoting-Rhizobacteria (PGPR) in plant health promotion under saline stressed environment

SNo	PGPR	Salt stress (mM NaCl)	Plant	Mechanisms	Inoculation impact on plant health	References
1.	*Klebsiella* sp.	100	*Avena sativa*	Modulate gene expression, Antioxidant activities	Significant reduction was observe in the antioxidant enzyme (SOD, POX), Proline, Malondialdehyde, and Electrolyte leakage and improvement shown in plant growth, relative water contents (RWC) in inoculated plants.	Sapre et al., 2018
2.	*Enterobacter* sp.	200	*Oryza sativa*	ACC deaminase activity	Inoculation decline CAT, SOD, POX, PPO, MDA and stress-induced ethylene. Rhizobacteria stimulate seedling germination and vigour index.	Sarkar et al., 2018
3.	*Bacillus amyloliquefaciens*	100	*Arabidopsis thaliana*	Stimulate stress responsive genes	Transcriptome profiling of gene associated with photosynthesis, phytohormone, SOS scavenging, Na^+ translocation, and osmo-protectants was differentially expressed and reduced salt susceptibility and stress facilitates adaptation.	Liu et al., 2017
4.	*Bacillus amyloliquefaciens*	100	*Arabidopsis thaliana,* *Zea mays*	Polyamine production	Spermidine productions by PGPR enhance GSH level through modulating glutamine synthetase and glutathione reductase gene expression and promote plant health.	Chen et al., 2017
5.	*Dietzia natronolimnaea*	150	*Triticum aestivum*	Modulating transcriptional machinery	Pointedly modulate ABA-signalling cascade genes (TaABARE and TaOPR1), SOS pathway genes (SOS1 and SOS4), Ion transporters (TaNHX1, TaHAK, and TaHKT1) and antioxidant enzyme (APX, MnSOD, CAT, POD, GPX, and GR) gene expressions under stress condition and promote plant vigour.	Bharti et al., 2016

6.	*Enterobacter* sp.	25,50, 75, 100	*Abelmoschus esculentus*	ROS-scavenging enzyme activity	Promote Okra plant through Up-regulated ROS pathway and antioxidant gene expressions.	Habib et al., 2016
7.	*Serratia* sp.	150-200	*T. aestivum*	ACC deaminase activity	Enhances plant growth, restored photosynthetic pigments, Manages Na^+/K^+ ratio and reduces toxic ion effect on wheat plant.	Singh and Jha, 2016
8.	*Alcaligenes faecalis*	100	*A. thaliana*	Volatiles production (Hexanedioic and butanoic acid)	Improvement observed in Cytokinin, Brassino-steroid, and Ethylene-defective mutant line in *A. thaliana* plant. Enhancement results in up-regulation of auxin and gibberellin pathways and confers salt tolerance in inoculated plant.	Bhattacharyya et al., 2015
9.	*Arthrobacter protophormiae*	200	*Pisum sativum*	ACC deaminase activity	PGPR significantly utilizes ACC and potentially decline stress induced ethylene level. Reduced stress resulted in improved plant weight, pigment content and diminish proline and lipid peroxidation.	Barnawal et al., 2014
10.	*Bacillus amyloliquefaciens*	200	*O. sativa*	Modulate gene expression profile	Modulating different stress regulated genes and protecting plant from saline toxicity.	Nautiyal et al., 2013
11.	*Alcaligenes* sp., *Bacillus* sp., *Ochrobactrum* sp.	150	*O. sativa*	ACC deaminase activity	Reduced ethylene level resulted in enhanced germination percentage, chlorophyll contents and plant health.	Bal et al., 2013
12.	*Brevibacterium iodinum*, *Bacillus licheniformis* *Zhihengliuela alba*	100, 150, 200	*Capsicum annuum*	Regulating stress ethylene synthesis	Reduce ethylene production by 44, 53 and 57% in inoculated plant and improves plant health and inflate salt tolerant index.	Siddikee et al., 2011

13.	*Bacillus* sp., *Enterobacter* sp., *Microbacterium* sp., *Paenibacillus* sp. *Burkholderia* sp.	0,30,60 gm L^{-1} NaCl	*T. aestivum*	Exopolysaccharide (EPS) producing potential	Increased plant biomass though inhibiting Na^{+} uptake by plant roots.	Upadhyay et al., 2011
14.	*Bacillus megaterium*	150	*Z. mays*	Modulate stress responsible genes pattern	PGPR enhance root hydraulic conductance (*L* value) which is significantly co-related with higher plasma membrane type two (PIP2) aquaporin amount. Increase in ZmPIP1:1 protein in plant leave promotes plant vigour.	Marulanda et al., 2010
15.	*Pseudomonas putida,* *Pseudomonas aeruginosa,* *Serratia proteamaculans*	1, 5, 10 and 15 dS m^{-1}	*T. aestivum*	ACC-deaminase activity	PGPR shown most promising for wheat plant, enhances plant height, root length, grain yield, 100 grain weight and straw yield up to 52, 60, 76, 19 and 67%, respectively, over control plant.	Zahir et al., 2009
16.	*Bacillus subtilis*	100	*A. thaliana*	Regulation of high-affinity K^{+} transporter (HKT1) gene	Regulation in HKT1 gene expression in plant parts enables plant to reduce stress induced damage and promote plant health.	Zhang et al., 2008
17.	*Azospirillum brasilense*	30, 50 and 80	*Lactuca sativa*	Transforming phytohormone levels	Treatments resulted in better seed germination, vegetative growth and biomass production.	Barassi et al., 2006
18	*Achromobacter piechaudii*	172	*Lycopersicum esculentum*	Synthesis of ACC-deaminase	PGPR effectively reduced stress ethylene in seedling and significantly improves water use efficiency (WUE). Enhancing (WUE) improves fresh and dry weights of seedlings and nutrient uptake.	Mayak et al., 2004

2.9 Organic Manure Amendments in Soil Salinity Removal

Fertile soils are finest natural resource is continuous depleting on human timescale. Excessive fertilizer application led to soil deterioration and land use changes often positively reduces agriculture farm dimensions (Singh et al., 2010). With rising agrochemicals impacts and conscious concerns about the negative environmental impacts organic matter is increasingly seen as an important source of nutrients in agricultural fields (Cavagnaro et al., 2014). Moreover, intensive animal husbandry practices have led to the accumulation of animal dung, which contain high amounts of organic matter and nutrients such as nitrogen and phosphorus which if not managed in a healthy and eco-friendly manner, can lead to environmental pollution (NRDC, 2005; Maji et al., 2017). Manure, composts, and vermin-composts, when applied to the soils in sufficient proportions, positive result deal with soil fertility rejuvenation and plant growth promotion (Murphy, 2014). Organic matter input exhibit positive correlation with soil microorganisms and effective in nutrient restoration, soil texture improvement, disease mitigation and positively restore and rejuvenation soil fertility. The ecological functions such as nutrient cycling and formation of soil aggregate can be facilitated by organic matter decomposition brought by soil microorganisms. The microbial community structure efficiently validates soil processes such as de-nitrification, nitrification and nitrogen fixation. This microbial biomass composed wide variety of plant growth promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi (AMF), actinomycetes, algae and protozoans. Organic amendments not only improve soil physico-chemical and nutrients status, but also enhance viability and the survival of novel bio-inoculant (Rashid et al., 2016). Plant-microbe-manure tripartite interactions may have vital role in sustainable stressed agricultural management since such associations play an imperative role in improving plant performance.

2.10 Combine Application of PGPR and Organic Manure Amendment in Paddy Crop Agriculture under Saline Soils

Organic farming technology associated with microbial bio-fertilizers and organic matter amendment will possess effective in sustainable crop productivity. The researchers around globe predict organic farming is an appropriate way to protect natural resource for future. Agriculture which is based on agriculturally important microbial bio-formulation technology for replenishing agrochemicals will be an emerging and eco-safe solution for climate. Bio-preparation of agriculturally important PGPRs and deploy them in association with manure, composts, biochar, and other organic source in agricultural fields will provide cost effective and environmentally safe solution for farmers as well as industry. The benefits of PGPRs in agriculture are reported by various researchers around globe. Various PGPRs promote plant through various plant growth promoting attributes as N_2-fixation, mineral solubilization, siderophore production and synthesis of various organic acids and phytohormone production (Hastemi and Maheshwari, 2018). PGPRs with such activities how’s neglect than that against land deteriorating agrochemicals which have been posed negative and sometimes irreparable threats to the environment. Organic amendments are economically and environmental friendly option to ease agrochemical aggregates and has potential to restore stressed soil (Singh et al., 2011). Organic source are vigorously perceived for soil health fitness, aeration capacity, conditioning soil through absorbing water contents, enhances root architecture and provide nutrients source for plants (Singh et al., 2010). Roots architecture is vastly biased by soil characteristics. Farmyard manure (FYM) is a vital partially composed organic source and extensively present their potentiality in agricultural fields. FYM generally acidic in nature and this property positively demolish pathogen and renovate soil fertility.

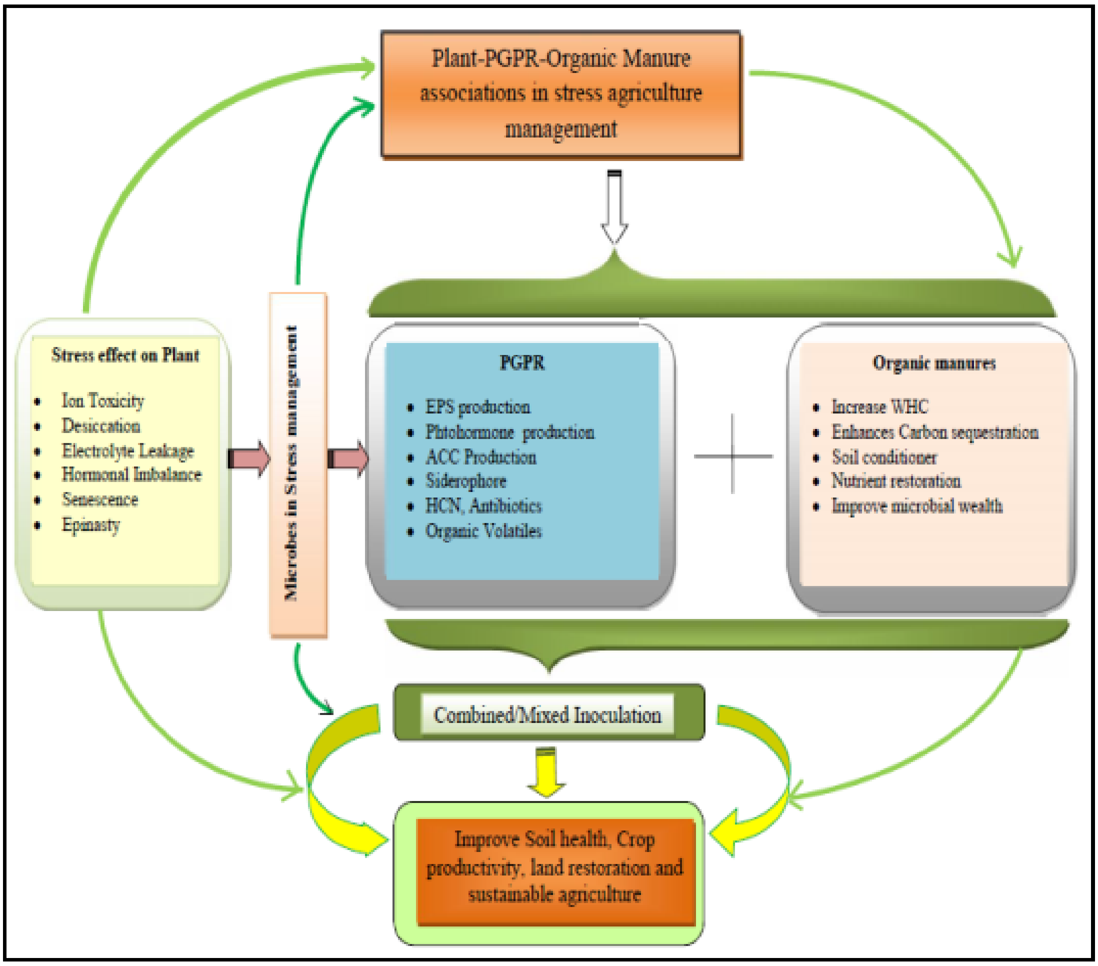


Figure 2.7 Plant-PGPR-Manure associations in stressed agriculture management

Supplementation of soils with FYM will enrich rhizospheric micro-flora and improve microbial activity (Singh et al., 2013; Cavagnaro, 2014). Improved microbial biomass structure attains plant growth, restore soil health and regenerate and restore degraded paddy ecosystem. Organic farming is the hope for environmental protection and ecosystem restoration; however, it has been arguedthat organic or agro-ecology farming systems are good for the environment, but these are often associated with lower yields when compared to the conventional farming systems (Seufert et al., 2012). It is therefore expected that enrichment of organic amendments with various effective and naturally occurring PGPR strains will be an effective, safe, viable, clean, and green technology to improve soil health and sustainability. With these observations in mind, present doctoral research work was endeavour to isolate efficient PGPR with salt tolerance capacity, develop bio-inoculant and deploys them in paddy agriculture and examines their effect on soil fertility, paddy plant health, productivity and antioxidant status under soil salinity.

CHAPTER 3

LOCATION OF SALINITY AFFECTED AREA, SOIL SAMPLING SITES, ISOLATION AND CHARACTERIZATION OF SALT TOLERANT PLANT GROWTH PROMOTING RHIZOBACTERIA

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LOCATION OF SALINITY AFFECTED AREA, SOIL SAMPLING SITES, ISOLATION AND CHARACTERIZATION OF SALT TOLERANT PLANT GROWTH PROMOTING RHIZOBACTERIA

3.1 Introduction

Soil salinity affects a large proportion of paddy crop productivity. Excess proportion salt ions disturb soil structure and retain soils fertility. High salt concentrations accumulate both Na^+ and Cl^- simultaneously, although the effects of these two ions may vary. During paddy cultivation in plane or flooded regions, the water added to the paddy soil is used by the crop plants, percolates downward or evaporates directly to the atmosphere. After evaporation, huge amount of salt accumulates on the soil surfaces. These salts effectively affect plant nutrient uptake, diminish microbial growth, crop yields and soil fertility. The Paddy plant under this stress condition demolishes and disturbs. Plant-microbe interactions play vital roles in the maintenance of plant and soil productivity under stress environment (Singh, 2015; Vimal et al., 2017). The PGPR promote plant growth through various mechanisms such as biofilm formation (Petrova and Sauer, 2016), phyto-hormones production (Barnawal et al., 2014), nutrients management (Singh et al., 2011) and antioxidants production (Upadhyay et al., 2012). Potential PGPR strains are also reported in the management of stresses by harmful radicals produced by plants during metabolism (Upadhyay et al., 2012). Based on the literatures available it is clear that PGPR applications in paddy fields, isolated from saline affected soils could be beneficial to alleviate the salinity stress of paddy crop plants. Therefore, in this research experiment salt tolerant PGPR strains from saline soils of salinity affected area of

Lucknow and adjoining districts will be isolated and identified. The most potential salt tolerant PGPR strain, having beneficial plant growth promoting attributes, after screening will be used as inoculants. The selected PGPR strain in combination with farm yard manure (FYM) will be applied in field condition to examine its impact on soil physico-chemical properties, antioxidant level and paddy plant growth parameters under soil salinity.

To survive various abiotic and biotic stresses, plants have evolved well developed antioxidative systems (Gill and Tuteja, 2010). Potential PGPR strains are also reported in the management of stresses by harmful radicals produced by plants during metabolism (Upadhyay et al., 2012). Antioxidants and antioxidative enzymes in plants, inoculated with PGP microbial agents, have been reported to scavenge the harmful radicals and minimize the stress induced damages (Khan et al., 2016). Antioxidants not only act as the compatible osmoprotectants but also serve as signalling molecules to modulate the osmotic balances of cell cytoplasm, and trigger the expression of specific genes, essential for the wellbeing of plant physiology and metabolism (Cui et al., 2016). It appears that PGPR could be an important group of beneficial microbes that can be exploited to alleviate the abiotic and biotic stresses in crop plants. Therefore, addition of salt tolerant microbial inoculants to the soil, could be the novel and cost-effective alternative to alleviate salinity stress in paddy crops. The isolation and identification of new salt tolerant PGPRs strains can be exploited to develop effective and viable bio-inoculants to enhance paddy production in salinity affected areas. There are scanty reports on application of PGPR inoculants to alleviate salinity stress, particularly the role of *Curtobacterium* sp. on paddy crop. Hence, the present study was conducted to investigate the efficacy of salt tolerant PGPR strains isolated from saline soils on paddy plant growth promotion under salt stresses. The most efficient isolate *C. albidum* SRV4 was selected on the basis of its in vitro PGP activity and 16S r-RNA gene sequencing for application. A greenhouse experiment was conducted to examine the effect of SRV4 inoculation on plant growth parameters and antioxidant activities of paddy plants under salinity.

3.2 Materials and Methods

3.2.1 Location of Soil Sampling Sites

The rhizospheric soil samples were collected from the salinity affected adjoining area of Lucknow districts.

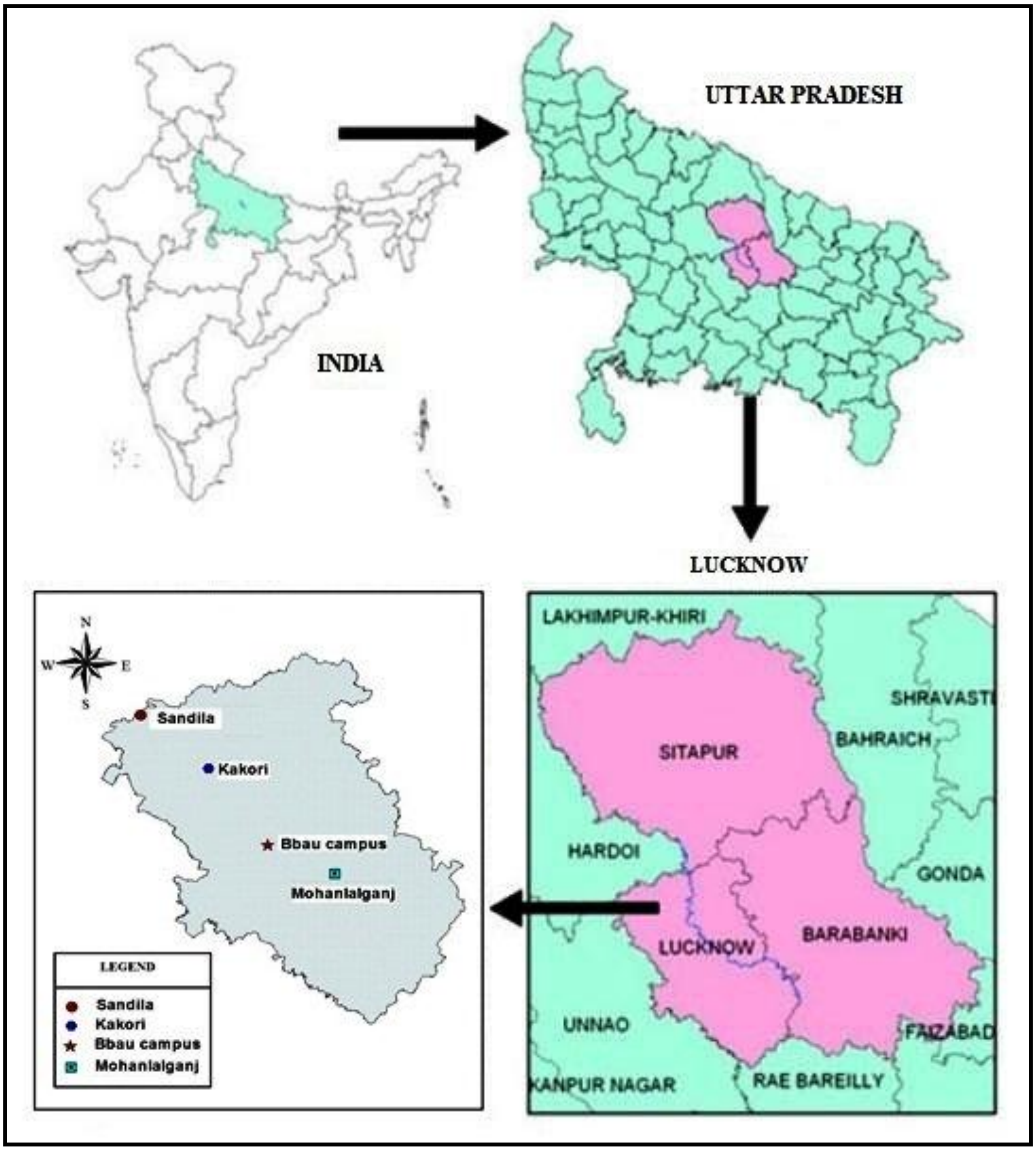

Figure 3.1 Location of salinity affected soil sampling sites

The first soil sample was collected from unfertile soils of Babasaheb Bhimrao Ambedkar University Campus, Lucknow. The soils of this site are unproductive, saline and electrical conductivity was found 6.7±0.04 ds m^{-1} (Singh et al., 2010). At this salinity level the growth of many plants are positively affected. Our second sampling site was Kakori, situated 14 km from Lucknow. Excess application of chemical fertilizer and improper irrigation techniques positively leads to soil salinity in this area (CSSRI Report, 2010). The third saline sampling site is Sandila, located around 55 km form the Lucknow. Unprecedented growth in Industrial sector in this area lead to salinity affected soils (Ghavri and Singh, 2012). Our fourth salt affected sampling sites is Mohanlalganj, Raebareli Road, is 25 km far away from Lucknow (Bharti et al., 2014).The electrical conductivity (EC) and pH of the collected soils were analysed immediately and transported to laboratory for microbiological analysis.

Table 3.1 Location of soil sampling sites of salinity affected area Lucknow district.

SNo	Sampling Sites	Coordinates
1.	BBAU campus, Lucknow	26°52'21"N, 80°57'20"E, 110 msl
2.	Kakori	26°86'94"N, 80°78'58"E, 121 msl
3.	Sandila	27°07'29"N, 80°51' 79"E, 142 msl
4.	Mohanlalganj	26°68'95"N, 80°98' 43"E, 130 msl

msl=mean sea level

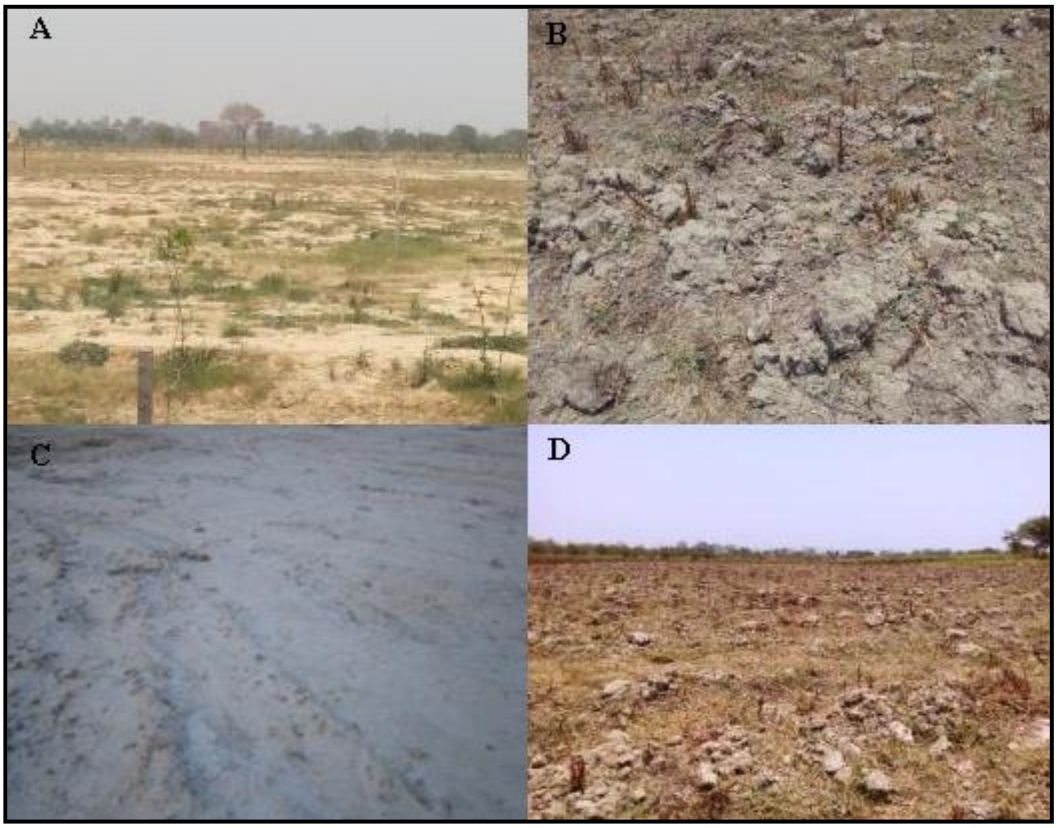

Figure 3.2 Soil sampling sites **(A)** BBAU **(B)** Kakori **(C)** Sandila **(D)** Mohanlalganj

3.2.2 Isolation of Salt Tolerant Rhizobacteria

The soil samples were serially diluted up 10^{-6} and plated on nutrient agar medium (Table 3.2) (up to1800 mM NaCl salt level) and incubated for 48-72 h at 28±2 °C. The pure rhizobacterial cultures were stored in 4°C for determination of PGPR activity, inoculum formulation and seed treatment.

Table 3.2 Nutrient agar medium composition

Ingredients	**g L^{-1}**
Peptone	5.000
NaCl	5.000
HM Peptone B	1.500
Yeast Extract	1.500
Agar	15.00

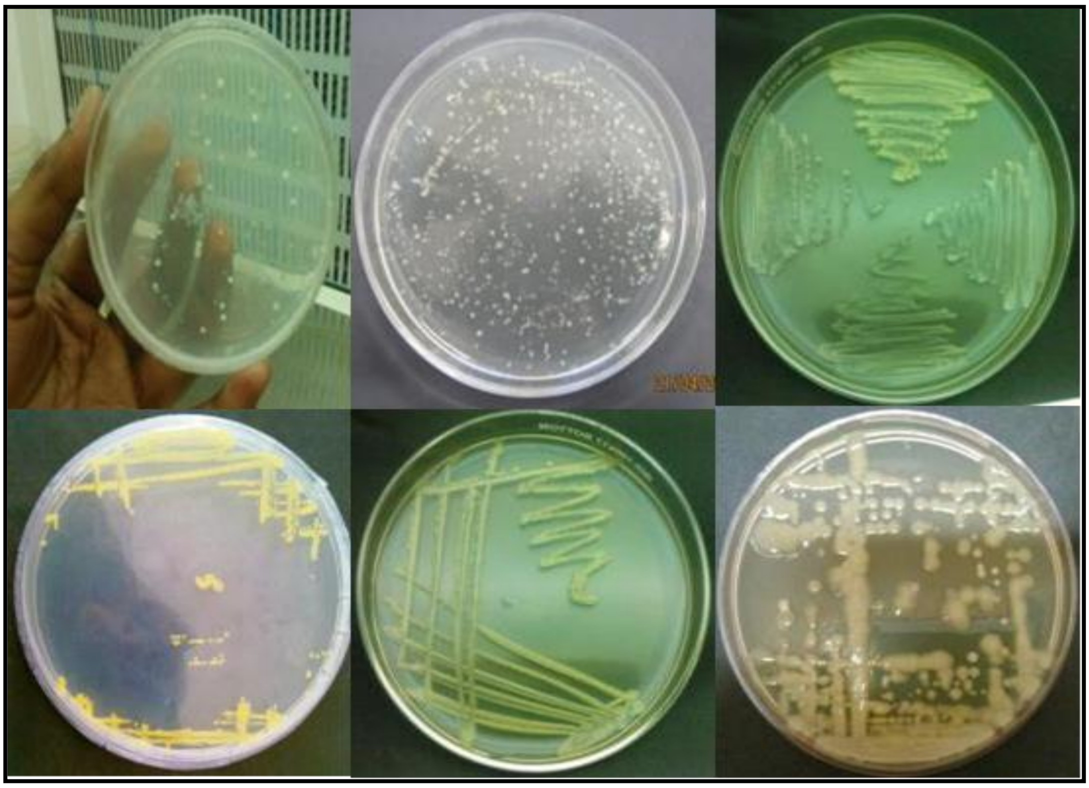

Figure 3.3 Isolation and purification of rhizobacterial strains

3.2.3 Plant Growth Promoting Attributes of Isolated Salt Tolerant Rhizobacteria

3.2.3.1 Nitrogen Fixation

Nitrogen fixation efficiency was determined with Jensen’s N free medium (Jensen, 1954). The isolated colonies were streaked on Jensen agar (Table 3.3) incubated for 48 h at 28±2 °C.

Table 3.3 Jensen’s N free medium composition

Ingredients	g L^{-1}
$C_{12}H_{22}O_{11}$	20.00
K_2HPO_4	1.0
$MgSO_4.7H_2O$	0.5
$FeSO_4.H_2O$	0.1
NaCl	0.5
Na_2MoO_4	0.005
$CaCO_3$	2.00
Agar	15.00

The bacterial growth was observed as qualitative evidence of N_2-fixation.

3.2.3.2 Phosphate Solubilization

The phosphate solubilization activity was determined according to Pikovaskaya (1948). The isolates were point inoculated on Pikovaskaya's agar medium (Table 3.4) and incubated at 28±2 °C for 5-7 days.

Table 3.4 Pikovaskaya agar medium composition

Ingredients	g L^{-1}
$(Ca_3)_2PO_4$	5.00
$(NH_4)_2SO_4$	0.5
NaCl	0.2
KCl	0.2
$MgSO_4.7H_2O$	0.1
$FeSO4.7H_2O$	0.001
$C_6H_{12}O_6$	10.00
Yeast Extract	0.5
Agar	15.00

The plates were observed for the zone of clearance around the bacterial colony which indicated solubilization of P. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone (Gaur, 1990).

3.2.3.3 Siderophore Production

Siderophore production efficiency of strains was carried on Chrom-Azurol S (CAS) medium.

Composition of CAS medium

Solution 1: 60.5 mg CAS dye +1mM $FeCl_3.6H_2O$ prepared in 10 mM HCl mixed with 50 mL doubled distilled water (DDW). Then, dissolved 72.09 mg Hexadecyltrimethylammonium bromide (HDTMA) in 40 mL DW and mixed with CAS solution.

Solution 2: 30.2 g piperazine-N,N′-bis (2-ethanesulfonic acid (PIPES) in dissolved in 800 mL DDW and maintained the pH 6.8 then added 15.00 agar g/L .

Solution 3: 70 mL solution containing glucose (2.00g), mannitol (2.00g), $MgSO_4.7H_2O$ (493.00mg), $CaCl_2$ (11.00mg), $MnSO_4.H_2O$ (1.17mg), H_3PO_4 (1.4 mg), $CuSO_4.5H_2O$ (0.04 mg), $ZnSO_4.7H_2O$ (1.2 mg) and $Na_2MoO_4.2H_2O$ (1.00 mg).

Solution 4: 30.00 mL of 10 % casamino acid Millipore filter sterilized.

Procedure for Preparing CAS Agar Medium

i. Added CAS indicator solution carefully with sufficient stirring so as to mix the ingredient without bubble formation.
ii. Poured the medium on sterilized Petri dishes and leave for overnight.
iii. A drop of raised broth culture were spotted on CAS plates and incubated at 28±2 °C for 72 h. Formation of orange halos around bacterial colonies showed that bacterial isolate was able to produce siderophore (Schwyn and Neilands, 1987).

3.2.3.4 HCN Production

Hydrogen cynide (HCN) production was determined with method of Lorck (1948) with modifications. Freshly grown cultures were streaked on Kings’B agar medium (Table 3.5)

Table 3.5 Kings’B agar medium composition

Ingredients	**g L^{-1}**
Protease peptone	20.00
K_2HPO_4	1.5
$MgSo_{4.}7H_2O$	1.5
Agar	15.00
Glycerol	15.00 mL

A filter paper saturated with 1% solution of picric acid and 2% Na_2CO_3 was placed on the lid of Petri dish. The Petri dish was sealed with paraffin tape and incubated at 28±2 °C for 4 days. Change in colour of filter paper from yellow to reddish brown was observed for cyanogenic activity.

3.2.3.5 Exopolysaccharide (EPS) Production

Exopolysaccharide (EPS) production was assayed qualitatively with method of Nicolaus et al. (1999). Bacterial strains were grown in a minimal media (Table 3.6) for 5 days at 150 rpm at 28±2 °C in 250 mL Erlenmeyer flasks each containing 100 mL broth supplemented with 5% NaCl.

Table 3.6 Minimal medium composition

Ingredients	**g L^{-1}**
$Na_3C_6H_5O_7$	3.00
KCl	2.0
$MgSo_4.7H_2O$	20.00
$MnCl_2.4H_2O$	0.036
$FeSO_4.7H_2O$	0.05
Casamino acids	7.5
Yeast Extract	10

Supernatants were collected after centrifugation at 10000×*g* for 10 minutes at 4°C. Cold absolute ethanol (3-fold) was then added drop wise under stirring and the formation of a precipitate was considered as positive for EPS production (Siddikke et al., 2011).

3.2.3.6 Indole-3-Acetic Acid Production

Indole-3-acetic acid (IAA) production was determined with slight modification in method of Gordon and Weber (1951). Pure bacterial strains were inoculated in Nutrient Broth medium (Table 3.7) and incubated in orbital rotary shaker (Scigenics-Biotech, India) for 72 h at 120 rpm at 28±2°C.. For quantitative estimation of IAA the procedures are as follows.

Table 3.7 Nutrient broth medium

Ingredients	**g L^{-1}**
Peptone	5.0
Beef Extract	3.0
NaCl	5.0

i. After 72 h, 2 mL of each culture was pelleted by centrifugation 6000×g for 4°C (Remi CPR-30 Plus) and the supernatant was discarded.

ii. Cell pellets were washed with 1 mL of PBS and re-suspended in phosphate buffer saline (PBS).

iii. About 1 mL of cell suspension (corresponding to a cell density of 10^7 cells/mL) was added to 10 mL of nutrient broth amended with tryptophan (100µg/mL) and incubated at 28±2 °C with continuous shaking at 120 rpm for 48 h.

iv. After incubation, 2 mL of bacterial culture was centrifuged at 12000×g for 10 minutes.

v. After this about 1 mL of supernatant was transferred to a fresh tube in which 100 µg/mL of 10 mM orthophosphoric acid and 2 mL of Salkowski's reagent (1 g of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) were added and incubated for 30 minutes in dark at room temperature.

vi. Development of pink colour indicates IAA production. Meanwhile, IAA concentration was quantified colorimetric method at 530 nm using spectrophotometer (Evolution 201 UV-Vis Spectrophotometer) and calculated by comparing with the standard curve prepared with crude IAA.

3.2.3.7 ACC Deaminase Production

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of isolates was performed according to Penrose and Glick (2003). All 29 isolates were screened for their ability to utilize 1-aminocyclopropane-1-carboxylate (ACC) (3mM) as a sole nitrogen source in Dworkin Foster (DF) minimal medium (Table 3.8) (Dworkin and Foster, 1958) according to method of Dell'Amico et al. (2005) with some modifications. The Quantitative estimation procedure is described below.

Table 3.8 Dworkin Foster (DF) minimal medium composition

Ingredients	**g L^1**
KH_2PO_4	4.0
Na_2HPO_4	6.0
$MgSO_4.7H_2O$	0.2
Glucose	2.0
Gluconic acid	2.0
Citric acid	2.0
Agar	15.00

Trace Elements

Elements	**mg L^{-1}**
$FeSO_4.7H_2O$	1.0
H_3BO_3	10.00
$MnSO_4.H_2O$	11.19
$ZnSO_4.7H_2O$	124.6
$CuSO_4.5H_2O$	78.22
MoO_3	10.00
$FeSO4.7H_2O$	1000.00

i. The culture conditions applied on DF salt minimal medium (Dworkin and Foster, 1958) alone was considered as a negative control and DF salt minimal medium with $(NH_4)_2SO_4$ (2.0g L^{-1}) as a positive control.

ii. The quantification of ACC deaminase activity was done by measuring the amount of α-ketobutyrate generated as a by-product of the reaction spectrophotometrically (UV-Vis Spectrophotometer SPECORD-50 Plus) at 540 nm (Barnawal et al., 2017).

iii. The activity of ACC deaminase production by rhizobacterial strain was expressed in nmol α-KB mg^{-1} protein h^{-1}.

3.2.4 Molecular Characterization of Rhizobacterial Isolate

The potent salt tolerant rhizobacteria strain was identified by 16s r-RNA gene (r-DNA) sequence analysis. Total genomic DNA was isolated from the log phase of bacterial culture. The quality and quantity of the DNA was analysed using agarose gel electrophoresis and Nanodrop ND-1000 Spectrophotometer. The universal primer 27F (5′AGAGTTTGATCCTGGCTCAG3′) and 1492R (3′ACGGCTACCTTGTTACGA-CTT5′) was used to partially amplify the 16s r-RNA encoding gene from the rhizobacteria (Edwards et al., 1989). Amplification was performed in 25 µL final volume containing 2 µL genomic DNA (100 ng), 1.5 µL each of forward and reverse primer, 2.5 µL 10×Taq polymerase buffer, 2.5 µL dNTPs, 0.4 µL Taq, 2 µL MgCl2 (250 mm), 1.25 µL $(CH_3)_2SO$ (5%), 0.80 µL BSA and 10.55 µL of Milli-Q water (MQ) (Weisburg et al., 1991). PCR conditions consisted of initial denaturation step at 95 °C (5 min), 31 amplification cycles of denaturation at 94 °C (1 min), annealing at 57.4 °C (1 min) and primer extension at 72 °C (2.30 min) followed by a final extension at 72 °C (15 min) with thermocycler (Awasthi et al., 2011). Aliquots of the PCR products were analysed in 1.5% (w/v) agarose gels by horizontal gel electrophoresis. The amplified product was purified with the PCR Clean-up Kit (Axygen) according to manufacturer’s protocol, and sequenced via universal primers and Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) on a *3130×l* Genetic Analyzer (Applied Biosystems, USA).

3.2.5 Sequence Analysis and Phylogenetic Tree Preparation

Sequence analysis was completed using the nucleotide BLAST (BLASTN) (http://www.ncbi.nlm.nih.gov/BLAST) on the National Centre of Biotechnology Information (NCBI) website. Phylogenetic and molecular relatedness of the 16S rRNA sequences of *C. albidum* SRV4 and its related species were shown by using software MeGAlign 6.0 and align using CLUSTALW. The nucleotide sequence data have been deposited with NCBI.

3.2.6 Application of Isolated Salt Tolerant Rhizobacterial Strain in Paddy Pot Experiment

3.2.6.1 Inoculum Preparation

The inoculum was developed according to method of Nandkumar et al. (2003) with some modifications. The detailed steps are given below.

i. To produce isolated bacterial strain inoculums, single colony of tested isolates was inoculated in 100 mL nutrient broth and incubated in orbital rotary shaker (Scigenics-Biotech, India) at 150 rpm for 48 h at 28±2 °C.
ii. The culture obtained at exponential phase was centrifuged at 6000×*g* (Remi CPR-30 Plus) for 10 minutes at 4 °C and bacterial cells were washed and re-suspended in 100 mL of 0.85% (NaCl) saline solution.
iii. The bacterial cell density was maintained up to 1×10^{8} CFU mL^{-1}. The enumeration and calculations of bacterial cell density were carried out following the drop method (Hoben and Somasegran, 1982). Briefly, 1 mL of pellet suspension was used to prepare 10 fold serial dilutions (10^{-2} -10^{-9}). In a final volume of 1 mL, about 10 µL of each dilution were placed on nutrient agar plates.
iv. After 24 h of incubation growth was recorded and calculations of cell density were done considering data from the last dilution in which bacterial growth was achieved.

3.2.6.2 Paddy Seed Treatments with Inoculum

For present study, the paddy seed cultivars (*Oryza sativa* L.) HUR 3-4 was procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, South Campus (Barkachha), Mirzapur, India. The seed treatment was done in following steps.

i. Seeds were washed with deionised water and treated with 2% sodium hypochlorite solution for 10 minutes for surface sterilization. The sterilize seeds were soaked in double volume of bacterial suspension (1×10^{8} CFU mL^{-1}) and kept at 28±2 °C on a rotary shaker (90 rpm) for 9 h to facilitate the penetration of the bacterial inoculums inside the seeds.

ii. Carboxy methyl cellulose (CMC) (2%) was added to the suspension as a sticker.

iii. After 9 h of inoculation, paddy seeds were dried in shade to use for further experiment. Solitary sterilized seeds were used as control in the experiment.

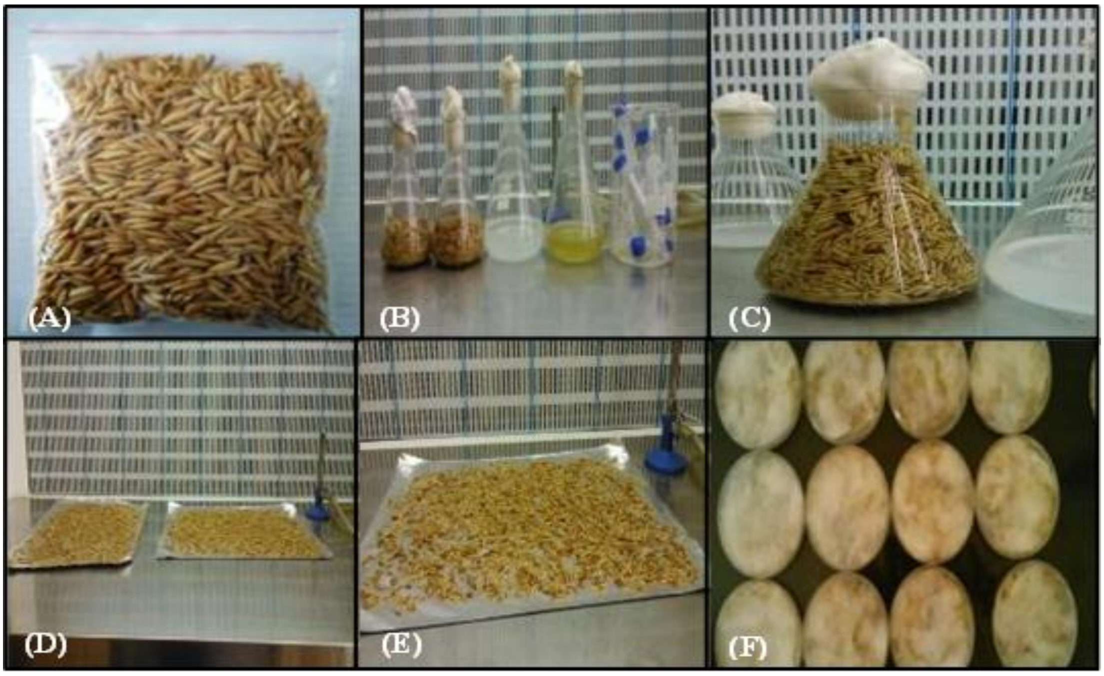

Figure 3.4 (A) Paddy seeds HUR-3-4 **(B)** Inocula development and seed treatment **(C)** Seeds dip in inocula **(D)** Treated seeds dried in laminar chamber **(E)** Bacterial coated seeds **(F)** Seeds germination

3.2.6.3 Paddy Pot Experiment

The pot experiment was conducted in earthen pots three times (triplicate) independently under greenhouse of Department of Environmental Science Babasaheb Bhimrao Ambedkar (Central) University Lucknow-India during July-September 2015. The treated as well as non-treated (control) paddy seeds were allowed to germinate in sterilized Petri dishes at 28±2 °C in BOD incubator for 12 h day/ night cycle (8 Watt fluorescent light) for 4 days. The germinated seedlings were transplanted in earthen pots containing sterilized soil (pH 7.1, EC 3.2 dS m^{-1}, Organic-C 0.14 %, organic matter 0.24%, available N 98 kg h^{-1}) and cultivated for 25 days in green house. After 25 days of successful cultivation, before transplantation, the root dipping mechanism was adopted to introduce the bacterial inoculums to the paddy seedling for efficient rhizobacterial attendance according to Nandkumar et al. (2003). Five healthy paddy seedlings per hills having equal height and three hills per pot were transplanted in earthen pots containing 9 kg of non-sterile field soil and farmyard-manure (FYM) in 3:1 ratio. A blanket application of NPK fertilizer (150:80:40 kg^{-1} soil) was also applied as a basal dose for plant successful development. The earthen pots with paddy plants treated with salt (NaCl) and without bacterial inoculation served as positive control, while pots without NaCl and bacterial culture were considered as negative control. To asses that impact of different doses of NaCl (0, 100, 200 and 300 mM) on paddy plants planted in earthen pots 750 mL of NaCl solutions were applied for each treatment. To achieve the final NaCl doses (100, 200 and 300 mM) for the respective treatment (except control) in earthen pots, the concentration of NaCl solution was increased gradually. After seven weeks, the paddy plants exposed to various salinity regimes were harvested on September 15, 2015 for growth parameter measurements and antioxidant enzymes analyses.

3.2.7 Inoculation Impact on Paddy Plant Growth Parameters and Antioxidant Activities

3.2.7.1 Measurement of Paddy Plant Growth Parameters

The plant samples having longest leaves were selected and sampled for the measurement of shoot height, root length with a meter scale.

3.2.7.2 Chlorophyll Contents

Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl a*+*b*) and carotenoids were examined spectrophotometrically with modified methods of Wellburn (1994). Fully expanded leave 0.5(g) samples were dipped overnight (12 h) in 85% acetone for the extraction of chlorophyll pigments. The supernatant taken were centrifuged at 6000×g for 5 minutes at 4°C (Remi CPR-30 Plus) and diluted with same concentration for spectrometric measurements. The pigment contents were calculated at absorbance of 452.5, 644, 663 nm alongside blank of untainted acetone (85%). *Chl a, b* and total chlorophyll and carotenoids were estimated using following formula:

$Chl\ a$ (mg g^{-1} FW) = $10.3\times A_{663} - 0.98\times A_{644}$

$Chl\ b$ (mg g^{-1} FW) = $19.7\times A_{644} - 3.87\times A_{663}$

Total chlorophyll = ($Chl\ a + Chl\ b$)

Total carotenoids (mg g^{-1} FW) = $4.2 \times A_{452.5} - [(0.0264\times Chl\ a) + (0.426\times Chl\ b)]$

FW = Fresh weight

A_{663} = Absorption at 663nm

A_{644} = Absorption at 644nm

$A_{452.5}$ = Absorption at 452.5nm

3.2.7.3 Membrane Stability Index

Membrane stability index (MSI) was measured in different salt treated plants following the method proposed by Pinhero and Fletcher, 1994. Fresh leaf samples (1 g) were crushed in small pieces and placed in 250 mL flask containing 100 mL deionized DDW. The flask mouth was sealed and placed in the water bath maintained at the constant temperature of 32°C. After 2 h, the electrical conductivity of the medium (EC_1) was measured. Then samples were autoclaved at 121°C for 30 minutes to complete tissues degradation and release of all electrolytes. Samples were cooled to 25°C and final EC_2 was measured. The MSI was calculated with following formula:

MSI (%) = $(1 - EC_1 / EC_2) \times 100$

EC_1 = Electrical conductivity before autoclave

EC_2 = Electrical conductivity after autoclave

3.2.7.3 Proline Contents

Proline content in paddy plants was determined by the method of Bates et al., 1973. Fresh leaves (0.5 g) were homogenized in 3.00 mL of 5% (w/v) sulfosalicylic acid. The homogenate was centrifuged at $10000 \times g$ for 10 minutes at 4°C (Remi CPR-30 Plus). Supernatant (500 µL) was treated with ninhydrin and glacial acetic acid (1:1, v/v). The mixture was boiled for 30 minutes at 100 °C and then the reaction was terminated on ice for 5 minutes. The reaction mixture was extracted with equal volume of toluene. The chromophore containing toluene was aspirated from the upper aqueous phase, warmed at room temperature and absorbance was read at 520 nm by UV spectrophotometer (Evolution 201 UV-Vis spectrophotometer). The proline content was determined by comparing with a standard curve using L-proline as standard (Sigma -Aldrich USA) and calculated on a fresh weight basis (µ mole g^{-1} FW).

µ mole proline/g FW = [(µg proline/mL×mL toluene)/115 µg/mole]/[(g sample)/5]

3.2.7.4 Na^+ and K^+

Na^+ and K^+ were determined with flame photometer (Systronics-130) after wet digestion of paddy plant (dried) materials with HNO_3-$HClO_4$ (3:1). The dried crust of plant material (1.00 g) was digested with 10.00 mL of digestion mixture and kept for overnight. After digestion, the flaks containing plant samples were placed on a hot plate and heated until the brown fumes turned white. On cooling, the digested samples were diluted with 50.00 mL of doubled distilled water and filtered through Whatman filter paper No 42. The filtrate was used for estimation of Na^+ and K^+.

3.2.7.5 Antioxidant Enzymatic Activities

After effective salt treatment, paddy plant leaves were sampled for the purpose of analyses of different enzymatic activities. About 0.2 g of fresh paddy leaves was homogenise in 2.00 mL of ice cold phosphate buffer (pH 7.8) containing 1 mM EDTA with sterilized mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4 °C. The supernatant was used for enzyme assays measurement according to Wang et al. (2012). The specific enzyme activity for all enzymes was expressed as unit mg^{-1}.

Catalase (CAT) (EC 1.11.1.6) activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm (Aebi, 1984). The reaction mixture (3 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 0.1% H_2O_2, and 0.1 mL of enzyme extract. The reaction was initiated by adding the enzyme extract. The decrease in H_2O_2 levels was determined by measuring the absorbance at 240 nm with spectrophotometer, and quantified using extinction coefficient (36 mM^{-1} cm^{-1}).

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was executed by measuring its effectiveness in inhibiting the photo-reduction of nitro-blue-tetrazolium (NBT) as described by Giannopolitis and Ries, 1977. The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 0.75 mM NBT, 0.02 mM riboflavin and 0.1 mL enzyme extract. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 20 W fluorescent lamps, which lasted for 10 minutes. Non-illuminated and illuminated reactions without enzyme extract served as calibration standards. The absorbance values of the reaction mixture and the blank control were measured at 560 nm with a spectrophotometer. One unit of SOD activity (U) was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate, and the results were expressed as unit mg^{-1} protein of FW. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photo-reduction.

Peroxidase (POX) (EC 1.11.1.7) capacity was based on oxidation of guaiacol using hydrogen peroxide (Zhang et al., 1996). The reaction was initiated by adding 20 µL of the enzyme extract to 3 mL of reaction mixture consisting of 100 mM phosphate buffer (pH 7.0), 20 µL of guaiacol solution, and 10 µL of hydrogen peroxide solution. The absorbance was measured at 470 nm at time points of reaction initiation and 5 minutes later with spectrophotometer. Enzyme activity was quantified based on the amount of tetra-guaiacol formed using the extinction coefficient (26.6 mM^{-1} cm^{-1}). A unit of peroxidase activity was expressed as µmoL mL^{-1} H_2O_2 decomposed per minute.

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was assayed according to Nakano and Asada, 1981. The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 mL enzyme extract. The reaction was initiated by addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 3 minutes. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}). One unit of APX was defined as 1 mM mL^{-1} ascorbate oxidized per minute.

Protein concentration in paddy plant was determined according to Bradford (1976) using bovine serum albumin (BSA) as a standard.

3.2.7.6 Statistical Analyses

The collected data from the experiment are expressed as the mean of three replicates ± SE each year of experiment. The analysis of variance (ANOVA) was performed to test the significance of the observed differences using windows-based statistical package for social science (SPSS) program (Version 20: IBM, Armonk, NY, USA). Data were compared with Duncan’s multiple range test (DMRT) at $p<0.05$.

3.3 Results

3.3.1 Isolation, PGPR Attributes and Molecular Characterization of Rhizobacterial Isolates

In the present study, about 110 rhizobacterial strains with different morphology was isolated from saline soils of semiarid regions of Sandila situated at Hardoi districts of Uttar Pradesh, India. About 29 promising bacterial strains having at least one PGPR traits *viz* N_2-fixation, P-solubilization, HCN, siderophore, EPS, IAA, and ACC deaminase activities were screened (Table 3.9). All 29 strains were evaluated at different doses NaCl concentrations (0-1800 mM).

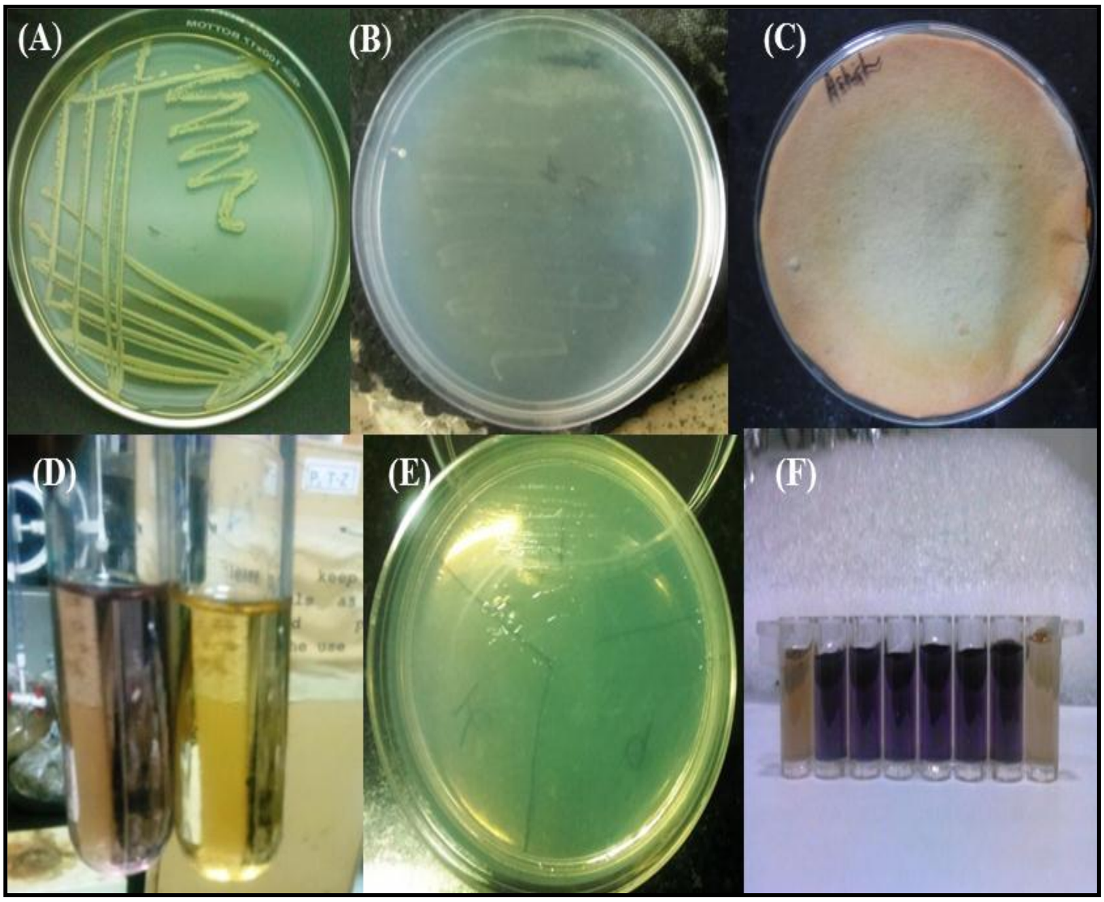

Figure 3.5 Plant growth promoting activities of isolated PGPR SRV4 strain (**A)** Strain SRV4, (**B)** N_2 fixation, **(C)** HCN production **(D)** IAA production, (**E, F)** ACC deaminase production)

Table 3.9 Promising PGPR isolates with different plant growth promoting activities

Isolates	**N_2Fixation**	**Phosphate Solubilization**	**Siderophore Production**	**HCN Producn**	**IAA Production**	**EPS Production**	**ACC deaminase**
SRV1	+	-	-	-	+	+	-
SRV2	+	-	-	+	+	-	-
SRV3	+	+	-	-	+	-	-
SRV4	+	-	-	+	+	+	+
SRV5	-	-	+	+	+	+	-
SRV6	-	-	+	+	+	-	-
SRV7	+	-	-	+	+	-	-
SRV8	-	+	-	-	+	+	-
SRV9	-	+	-	-	+	+	-
SRV10	-	+	-	+	+	+	-
SRV11	+	-	-	-	+	-	-
SRV12	+	-	+	-	+	-	-
SRV13	-	+	+	-	+	-	-
SRV14	-	-	+	+	+	-	-
SRV15	-	+	-	-	+	-	-
SRV16	+	-	+	-	+	+	-
SRV17	+	+	+	-	+	-	-
SRV18	-	+	-	+	+	+	-
SRV19	+	-	-	-	+	+	-
SRV20	+	-	-	+	-	+	-
SRV21	+	+	-	+	+	-	-
SRV22	-	+	-	+	+	-	-
SRV23	+	-	-	-	+	+	-
SRV24	-	-	+	+	-	-	-
SRV25	-	-	+	-	-	-	-
SRV26	+	-	+	+	+	+	-
SRV27	+	-	-	-	+	+	-
SRV28	-	+	+	+	+	-	-
SRV29	-	-	+	+	+	-	-

(HCN=Hydrogen cyanide, IAA=Indole acidic acid, EPS=Exopolysaccharides, ACC=1-aminocyclopropane-1-carboxylate)

Out of 29, only one bacterial strain (Gram positive) showed positive results for the N_2-fixation, EPS, IAA (16.1 ± 0.5 µg mL^{-1}), HCN production and ACC deaminase (296 ± 11.2 nmol α-KB mg^{-1} protein h^{-1}) activity.

Table 3.10 Quantitative IAA and ACC deaminase traits of selected salt tolerant rhizobacteria strain

Strain	**Gram reaction**	**IAA activity µg mL^{-1}**	**ACCD activity (nmol α KB mg^{-1} Pr. h^{-1})**	**Identified as**	**Genbank accession no**	**Source**
SRV4	+	16.1±0.5	296±11.2	*Curtobacterium albidum*	KX 817280	Saline soil

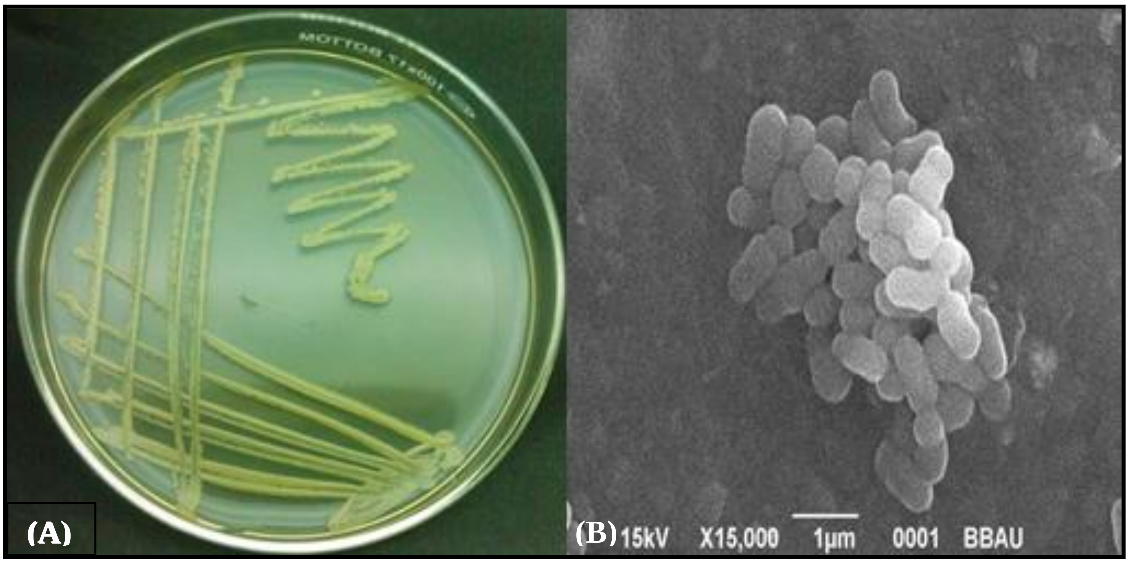

Figure 3.6 (A) SRV4 strain on nutrient agar **(B)** SEM photomicrograph of SRV4 strain

Molecular characterization, based on the nucleotide BLAST (BLASTn) analysis of 16S rRNA sequence indicated that the bacterial isolate has closest relationship with *C. albidum* with 98 % similarity, and named as SRV4 strain. To further confirm the results of BLASTn, phylogenetic analysis of SRV4 strain was performed with some other species of *Curtobacterium* and a strain of *C. albidum* DSM20512. Results revealed its relatedness with several other strains of *Curtobacterium* sp. (Figure 3.7). The sequence data of *C. albidum* SRV4 has been deposited to the GenBank nucleotide sequence database with the accession number KX 81071.

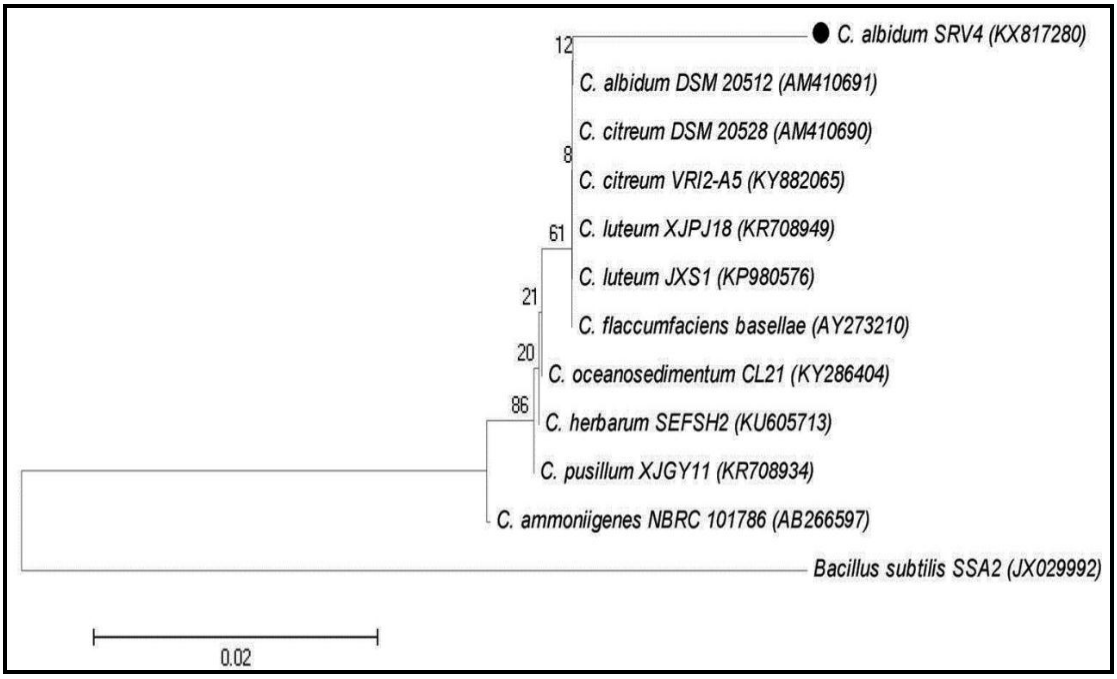

Figure 3.7 Phylogenetic relationship of isolated rhizobacterial strain SRV4 with other closely related bacterial strains based on 16s rRNA gene sequence available from National Center for Biotechnology Information (NCBI) database library. (The branching pattern was generated by neighbour-joining method from the Molecular Evolutionary Genetics Analysis (MEGA) version 6 software package (Tamura et al., 2013), using *Bacillus subtilis* SSA2 as an out group member).

3.3.2 Effect of Isolated SRV4 strain on Paddy Plant Growth Parameters in Pot Experiment

The results related to different saline regimes on paddy plant growth parameters in presence and absence of SRV4 strain has been depicted in Table 3.11. The addition of salt (NaCl stress) disturbed paddy growth performance. Across different NaCl treatments, highest reduction in paddy plant growth parameters was noted at 300 mM NaCl, followed by 200 mM and 100 mM NaCl compared to control (without NaCl treatment). But, inoculation of SRV4 isolate showed enhancement (8.25 to 33.2 %) in paddy plant growth parameters and variations due to treatments were significant ($P <$ 0.001 to 0.005). Inoculation of SRV4 strain along with different doses of NaCl treatments to the paddy plants enhanced plant height (from 10.37 to 19.01%), root length (from 11.72 to 23.51 %), tiller number (from 8.25 to 33.2 %) and dry weight (from 8.65 to 22.89 %) compared to sets treated with respective doses of NaCl only.

Table 3.11 Effect of SRV4 strain on paddy plant morphology under NaCl treatments. Total samples for each study parameter analysed was N=24 (8 treatments × 3 replicates). The value for each parameter given is means of three independent experiments ±SE. The superscript letter denote significant difference between means at $p<0.05$.

Treatments	**Plant height (cm)**	**Root length (cm)**	**Tillers (plant^{-1})**	**Plant dry weight (g plant^{-1})**
Control	55.36±1.27^{a}	18.56±1.24^{a}	5.33±0.66^{a}	18.16±1.41^{a}
Control+SRV4	68.20±1.58^{e}	23.26±1.63^{e}	7.33±0.33^{e}	23.03±1.54^{e}
100mM	52.60±1.92^{b}	16.03±1.17^{b}	5.0±0.57^{a}	16.73±1.88^{b}
100mM+SRV4	62.60±1.93^{f}	19.8±1.24^{f}	6.66±0.33^{f}	20.56±1.49^{f}
200mM	47.26±1.43^{c}	13.24±1.00^{c}	4.66±0.33^{b}	14.46±1.06^{c}
200mM+SRV4	54.83±1.76^{g}	15.86±1.41^{g}	5.66±0.88^{g}	17.3±1.36^{g}
300mM	41.16±1.28^{d}	10.80±1.15^{d}	4.0±0.57^{b}	10.4±1.76^{d}
300mM+SRV4	45.43±1.39^{h}	12.06±1.23^{h}	4.33±0.33^{h}	11.3±1.08^{h}
Significance level	$P < 0.001$	$P < 0.001$	$P = 0.006$	$P < 0.001$

3.3.3 Effect of SRV4 strain on Chlorophyll Pigments, Osmolytes and Antioxidant Enzymes under Pot Experiment

The selected photosynthetic pigments of paddy plants varied significantly ($P<0.001$) due to various treatments (Table 3.12). When the paddy plant was treated with different doses of NaCl (100 mM to 300 mM NaCl concentration) about 13.63 - 44.81 % reduction in MSI was noted in Figure 3.8 (A). However, SRV4 treated sets exhibited MSI enhancement by 23.31 % (100 mM), 29.36 % (200 mM) and 41.91 % (300 mM) NaCl concentration compared to respective controls (only NaCl treatment).

Table 3.12 Effect of NaCl stress and SRV4 strain on chlorophyll contents in paddy plants. Total samples for each study parameter analysed was N = 24 (8 treatments × 3 replicates). The value for each parameter given is mean of three replicates ±SE. The superscript letter denote significant difference between means at $p<0.05$.

Treatments	***Chl* a (mg g^{-1} FW)**	***Chl* b (mg g^{-1} FW)**	**Total *Chl* (mg g^{-1} FW)**	**Carotenoid (mg g^{-1} FW)**
Control	1.73±0.019^{a}	3.48±0.063^{a}	5.21±0.061^{a}	0.881±0.025^{a}
Control+SRV4	2.07±0.030^{e}	4.76±0.027^{e}	6.83±0.010^{e}	0.988±0.012^{e}
100mM	1.32±0.021^{b}	3.19±0.205^{a}	4.51±0.193^{b}	0.726±0.070^{b}
100 mM+SRV4	1.53±0.047^{f}	3.83±0.093^{f}	5.37±0.082^{f}	0.946±0.085^{e}
200mM	1.09±0.056^{c}	2.80±0.019^{b}	3.90±0.041^{c}	0.668±0.031^{b}
200 mM+SRV4	1.23±0.014^{g}	3.28±0.154^{g}	4.51±0.145^{g}	0.775±0.024^{f}
300mM	0.82±0.088^{d}	2.26±0.075^{c}	3.08±0.143^{d}	0.492±0.029^{c}
300 mM+SRV4	0.89±0.081^{h}	2.56±0.023^{h}	3.46±0.068^{h}	0.531±0.002^{g}
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Across different NaCl doses alone, or in combination with SRV4, proline content in paddy plants enhanced significantly and ranged from 2.12 to 4.07 µ mole g^{-1} fresh weights (Figure 3.8(B)). The addition of SRV4 strain indicated further enhancement in proline content ranging from 7.38 to 11.11 %. The increased accumulations of proline content in paddy plants due to application of strain SRV4 proved its efficacy to induce systemic tolerance in paddy plants against salt stress.

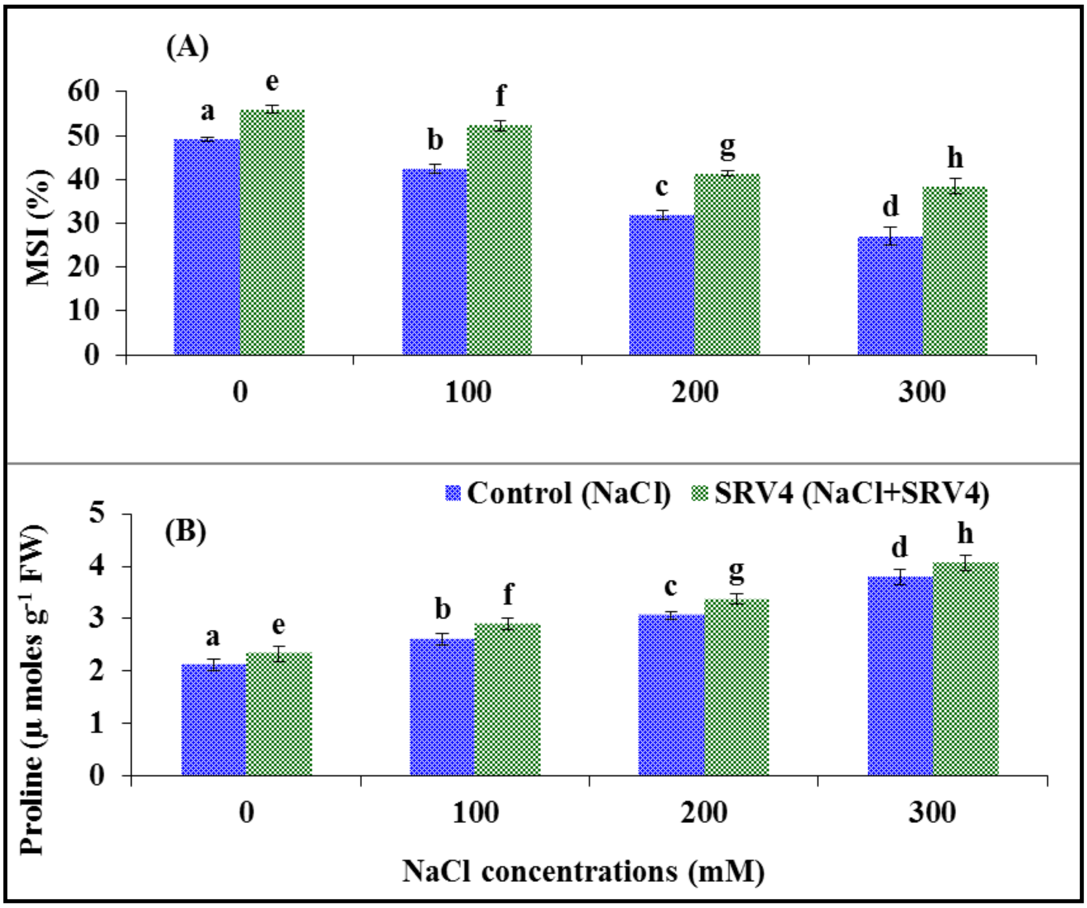

Figure 3.8 SRV4 inoculation impact on **(A)** membrane stability index (MSI) **(B)** Proline content in paddy plants

Figure 3.9 (A) shows elevation in Na^+ accumulation by paddy plants (1.11 to 2.03 mg g^{-1} FW) from 100 to 300 mM. But the application of respective NaCl doses with SRV4 strains reduced Na^+ accumulation (9.0 to 27.09 %). NaCl treatments declined K^+ in paddy plants (Figure 3.9 (B)) or could disturb cytoplasmic Na^+/K^+

homeostasis. However, SRV4 isolate application with different doses of NaCl enhanced K^+ accumulation (ranged from 11.22 to 39.21 %).

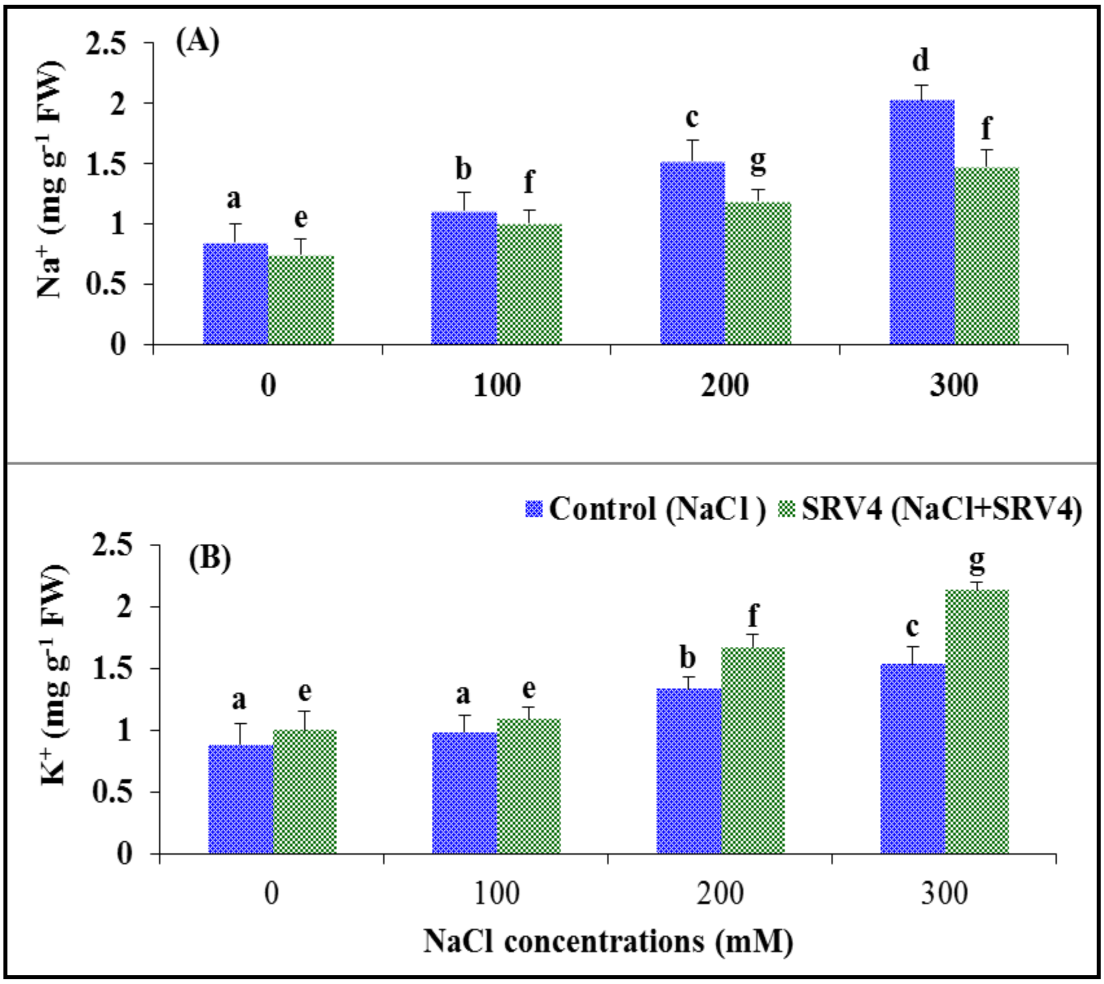

Figure 3.9 Na^+ /K^+ content in paddy plant under soil salinity

The CAT was enhanced with the increasing NaCl dose (Figure 3.10 (A)). The increase in CAT activity across different NaCl doses ranged from 21.10 to 44.95%. Inoculation of SRV4 with different NaCl doses further enhanced CAT activity (ranging from 27.27 to 51.26 %) compared to respective controls. Similar trends for the enhancement in SOD (Figure 3.10 (B)), POX (Figure 3.10 (C)) and APX activity (Figure 3.10 (D)) was also noted for different doses of NaCl alone or in combination with SRV4 compared to respective controls.

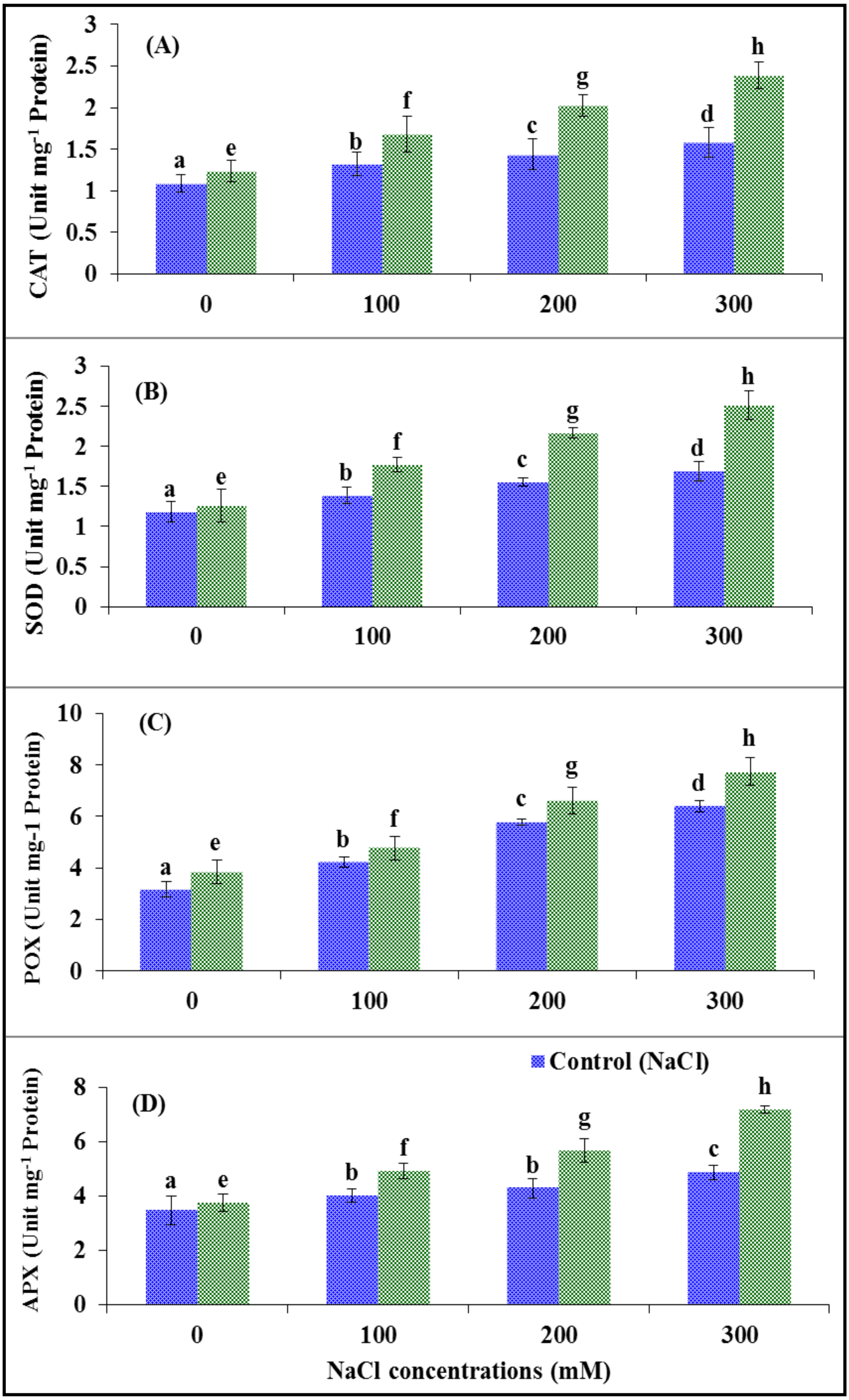

Figure 3.10 Antioxidant enzyme activities in paddy plant

3.4 Discussion

In this investigation, a comprehensive screening of native bacterial population from saline soils was carried out for their plant growth promoting attributes and ability to survive at different salt concentrations. The higher EC value (6.8dSm^{-1}) of the collected soil samples indicated saline nature of the soil. Therefore, the isolated bacterial population, having ability to grow and survive in saline soils, may be considered as salt-tolerant. Out of 29 PGPRs, the most potent SRV4 isolate having salinity tolerance (up to 1500mM NaCl) and plant growth promoting attributes, was selected for inoculation to the paddy plants.

High soil salinity has been considered as most determinant adverse and stress condition, affecting paddy crop productivity by affecting plant physiology and growth parameters (Upadhyay et al., 2011). However, the recent investigations suggest that inoculation of salt-tolerant microbial agents to saline soils improved crop plant growth by alleviating the salt stress (Singh et al., 2016; Vimal et al., 2017). Therefore, present study investigated the influence of an efficient salttolerant rhizobacterium C. albidum SRV4 application on plant growth parameters, osmolytes and antioxidant enzymes levels of paddy under various salinity levels.

Inoculations of PGPRs to the soils have improved crop plant growth by their ability to solubilize phosphate, production of various low molecular mass compounds (phytohormones) and/or antioxidants, accumulation of osmoprotectants such as proline, and balancing the Na^+/ K^+ ratio (Upadhyay et al., 2011; Upadhyay and Singh, 2015). Salt stress due to Na^+ accumulation can induce nutrient and osmotic imbalance in plants that can lead to stunted paddy plant growth (Khan et al., 2016). The data of present study showed that accumulation of Na^+ in paddy plants declined when the crop was grown with the inoculation of SRV4 bacterium. An improved EPS production by SRV4 strain could be one the major reasons to help paddy plants to overcome salinity stress by reducing the supply of Na^+ ions to plants (Upadhyay et al., 2011). Further, the inoculation of SRV4 indicated an elevated level of K^+ accumulation in the paddy plants, might be the additional reason in the mitigation of salt stress imposed by high salinity. It is reported that plant survives excessive salt concentrations through Na^+ omission, osmotic stress tolerance, and tissue Na^+ adaptation (Munns and Tester, 2008). It is assumed that in paddy plants, Na^+/K^+ homeostasis was well controlled due to inoculation of SRV4 strain. Several reports also demonstrated that a PGPRs inoculation manages the K+/Na+ ratio and therefore, salinity tolerance in crop plants (Nadeem et al., 2013). A statistically significant enhancement in paddy plant growth parameters following co-inoculation with SRV4 bacterium (Table 3.11), compared to un-inoculated controls indicates that this bacterium, like other PGPRs (Upadhyay et al., 2011), also has the potential to control deleterious effects of salinity stress on growth of paddy plants.

The photosynthetic pigments such as chlorophyll a, b, total-chlorophyll and carotenoids decreased significantly ($P < 0.001$) in the paddy plants at higher doses of NaCl treatments. But due to inculcation of SRV4 strain, a significant increase in photosynthetic pigments was noted. The decrease in photosynthetic pigment contents at higher NaCl doses may be due to salinity stress developed by higher concentrations of Na^+. A higher dose of NaCl treatment has been noted to decline the photosynthetic pigments (chlorophylls and carotenoids) status. Decline in photosynthetic pigments was maximum at 300mM NaCl. However, application of SRV4 strain with different doses of NaCl showed efficiency of pigments restoration compared to NaCl treatments alone. The restoration of pigments shows the ability of SRV4 strain to induce systemic tolerance under NaCl stress.

Excess amount of Na^+ as well as Cl^- may disturb the ionic equilibrium of the cell, leading to damage of membrane stability. The higher carotenoids (non-enzymatic antioxidants) generation due to addition of SRV4, can serve as the accessory pigment to protect the various chlorophyll pigments under salinity stress.

The higher proline content generation in response to elevated salinity, has also been reported as the powerful antioxidant in managing oxidative adjustments and protects intracellular macromolecules against dehydration (Upadhyay and Singh, 2015). An improved production of various photosynthetic pigments in PGPRs inoculated paddy seedlings under salt stress as reported by Kumar et al. (2017) supports the findings of this investigation.

As the data of present study indicated that the antioxidative enzymatic activities such as CAT, SOD, POX and APX increased in paddy plants with increase in NaCl dose. Plants have developed defence enzymatic systems to alleviate and re-nature the damages caused by salinity mediated oxidative stress (Upadhyay et al., 2012). In general, the increase in the antioxidative enzyme activities in response to stress conditions such as fly ash (FA) treated soil of paddy fields was found to be directly correlated with the levels of heavy metals accumulation in the leaves of paddy plants (Singh and Pandey, 2013). In the present experiments, an enhanced antioxidative enzymes production in paddy plants, possibly could be to neutralize the adverse impact of higher salinity stress and is in conformity with the results of Gill and Tuteja (2010) who demonstrated higher antioxidant ability of plants under abiotic stress. These results suggest that higher antioxidative enzyme requirement by paddy plant at higher salinity levels, could play as important role to protect plants against oxidative injury.

3.5 Conclusions

The aim of the present investigation was just a preliminary study to isolate and identify the efficient PGPR strains from the saline soils that could be evaluated from pot to field conditions to improve the growth and yield of paddy crop under salinity stress. The pots inoculated with SRV4 strain increased paddy plant growth compared to the non-inoculated controls under various doses of salt concentrations. Based on the above results, this study suggests that inoculation of *C. albidum* SRV4 had significant impact on paddy growth due to their plant growth promoting attribute under salinity stress. The data showed that salinity toxicity at higher doses of NaCl may cause harmful osmotic disturbance in the paddy plants, and osmolytes and antioxidative enzymes could play a defensive role against damages due to salinity stresses. However, inoculated plants show significantly better plant health under saline stresses. To the best of our knowledge, this may be the first report with reference to the *C. albidum* inoculation in soils to alleviate salinity stress and improve paddy growth parameters. To validate pot experiment we further conducted two year paddy crop experiment under saline soils with *C. albidum* SRV4 in association of farmyard manure and examine inoculation impact on soil physicochemical properties, crop yield and antioxidant activities.

CHAPTER 4

EXPERIMENTAL DESIGN, APPLICATION OF TREATMENTS AND PADDY CULTIVATION IN FIELD CONDITION

CHAPTER 4

EXPERIMENTAL DESIGN, APPLICATION OF TREATMENTS AND PADDY CULTIVATION IN FIELD CONDITION

4.1 Introduction

Soil ecosystems surrounded by different climatic variables is affected by a variety of environmental stresses. Natural and anthropogenic sources potentially affect the soil fertility and its functioning. Salt salinity is one of the principal edaphic features for declining crop productivity in salt affected soils throughout the world (Vimal et al., 2017). Salts in excess proportion disturbed the ionic equilibrium and causes oxidative stress (Nadeem et al., 2014; Kang et al., 2014). However in greater concentration salt diminish physical, chemical, microbiological soil microbial process and finally affects the plant growth. Enhancement in electrical conductivity due to soil salinity adversely affects the soil texture, bulk density, aeration capacity, permeability and essential nutrient contents (Tejada and Gonzalez, 2005). Thus, sustainable management of agricultural fields affected by soil salinity a need of the hour for future crop production enhancement.

In the past few decades the research has reversed on their traditional solutions for protecting the most important natural resource soil. The first green revolution which reliance on hybrid varieties of crops for elevated crop yield in association of modern agricultural technologies, irrigation and heavy doses of chemical fertilizer harshly disturb the soils health and led to ecosystem disturbances. Uses of organic amendments such as mulch, manures, composts, biochars, etc. have been given serious attentions around the Globe for sustainable reduction and remediation of disturbed agro-ecosystems. Generally, the slightly acidic nature of composts/FYM has been reported effective in soil nutrient mineral solubilization, conductivity reduction and soil stabilization (Singh et al., 2010). Decline in Na^+ leaching, exchangeable sodium percentage and electrical conductivity are potentially associated with organic matter amendment (Singh et al., 2011). Several exogenous organic matters such as FYM and composts are effectively enhanced the soil organic carbon (SOC) levels (D’Hose et al., 2014; Vanden-Nest et al., 2014). Andriamananjara et al. (2016) examined addition of FYM in paddy crop agriculture in highly weathered soils and concluded that FYM are effective in phosphorus (P) availability to plants. Thus, FYM may be considered as potential and possible amendments to restore and rejuvenate the soil productivity of stressed paddy agro-ecosystems.

The PGPR, an efficient group of microbes, can efficiently promote plant growth under abiotic and biotic stress environments. Several PGPR strains with their unique characteristics have been found to improve the productivity of *Oryza sativa* (Nautiyal et al., 2013), *Triticum aestivum* (Upadhayay and Singh, 2015) and *Lycopersicum esculantum* (Mayak et al., 2004; Tank and Saraf, 2010) under soil salinity. We also isolated and examined the use of *C. albidum* strain SRV4 under greenhouse pot experiment on paddy plant health under salinity stresses.

Since reports related to impact of FYM in combination with salt tolerant PGPRs application on soil physico-chemical characteristics in saline paddy filed are lacking therefore, this study was conducted to assess the impact of salt tolerant PGPR *C. albidum* SRV4 strain + FYM on soil properties under field conditions. We hypothesize that FYM in combination with salt tolerant *C. albidum* SRV4 strain will be effective in improving soil conditions and soil salinity amelioration of paddy fields. The improved soil conditions due to FYM and plant growth promoting attributes of *C. albidum* SRV4 strain would be beneficial to remove the stresses because of salinity and ultimately a better soil health and its functioning.

4.2 Materials and Methods

4.2.1 Experimental Design of Paddy Field

The field experiment was conducted for two successive paddy crop cycles in the year 2015 and 2016, at field experimental station, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India.

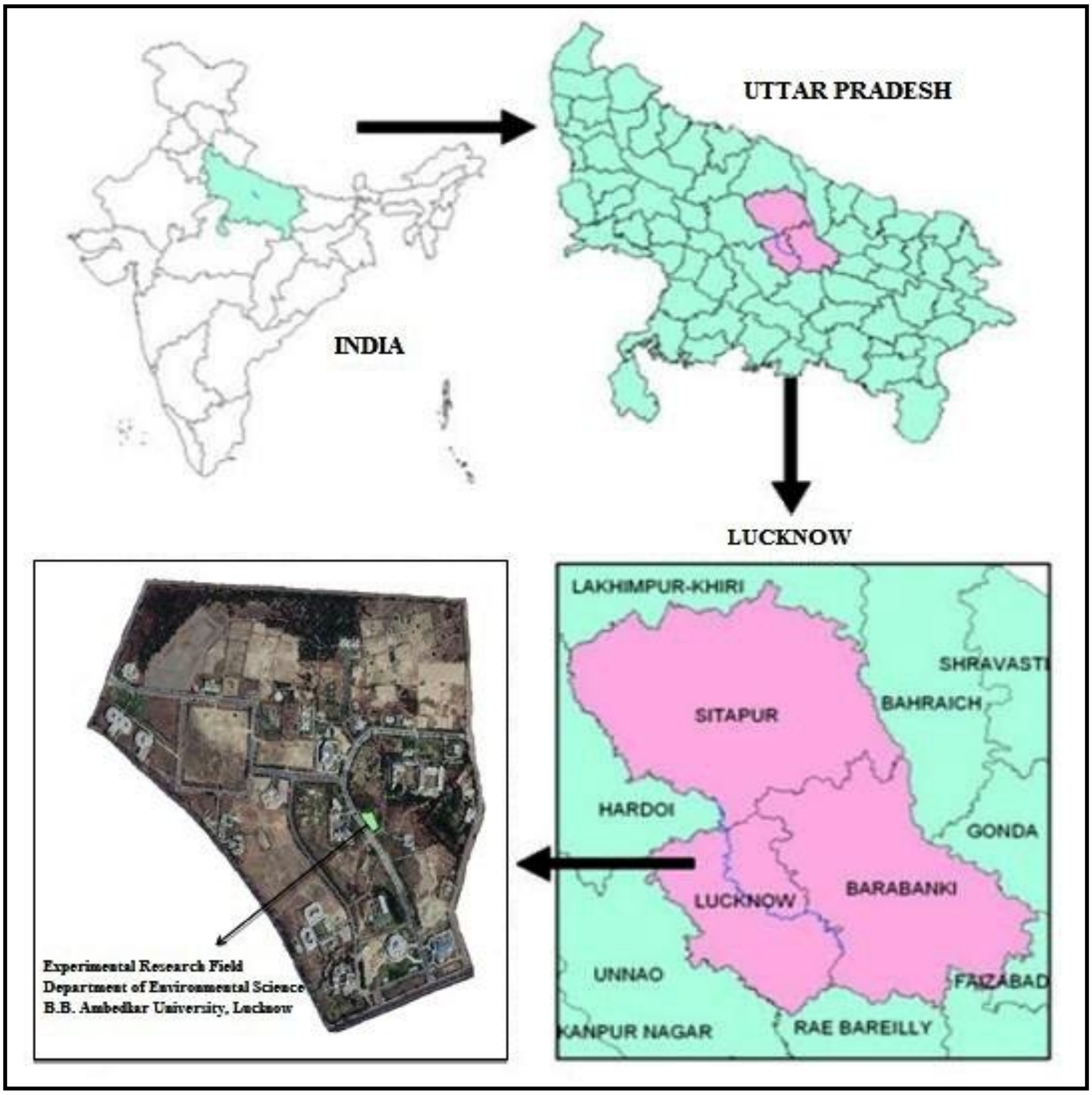

Figure 4.1 Location of experimental research field

The farm field was harrowed and disc ploughed by a cultivator (Tractor) in month of June for both the years 2015 and 2016. After ploughing, the weeds and other residues were removed and field was levelled manually using hand hoes. Therefore, total 12 experimental plots (each having 3m×2m dimensions) were established in completely randomized block design (CRBD) with each treatment having three replications. To differentiate each experimental plot a strip of 30 cm was made to prevent the possibility of nutrients and microbial exchange in soils among experimental plots. The FYM (procured from market) was crushed and incorporated superficially in the soil manually homogenous mixing by hands. The physico-chemical characteristics of FYM, applied in paddy experimental filed had pH 6.1, BD 1.21, organic-C 23.46%. The FYM amended in experimental plots showed slightly acidic in nature. A blanket application of NPK fertilizer (160:80:40 kg ha^{-1}) was also served as basal dose for paddy crop establishment for both the years. For both the study years (2015 and 2016), half of the suggested dose of N was applied at the time of transplanting and other half during panicle initiation during paddy crop cycle.

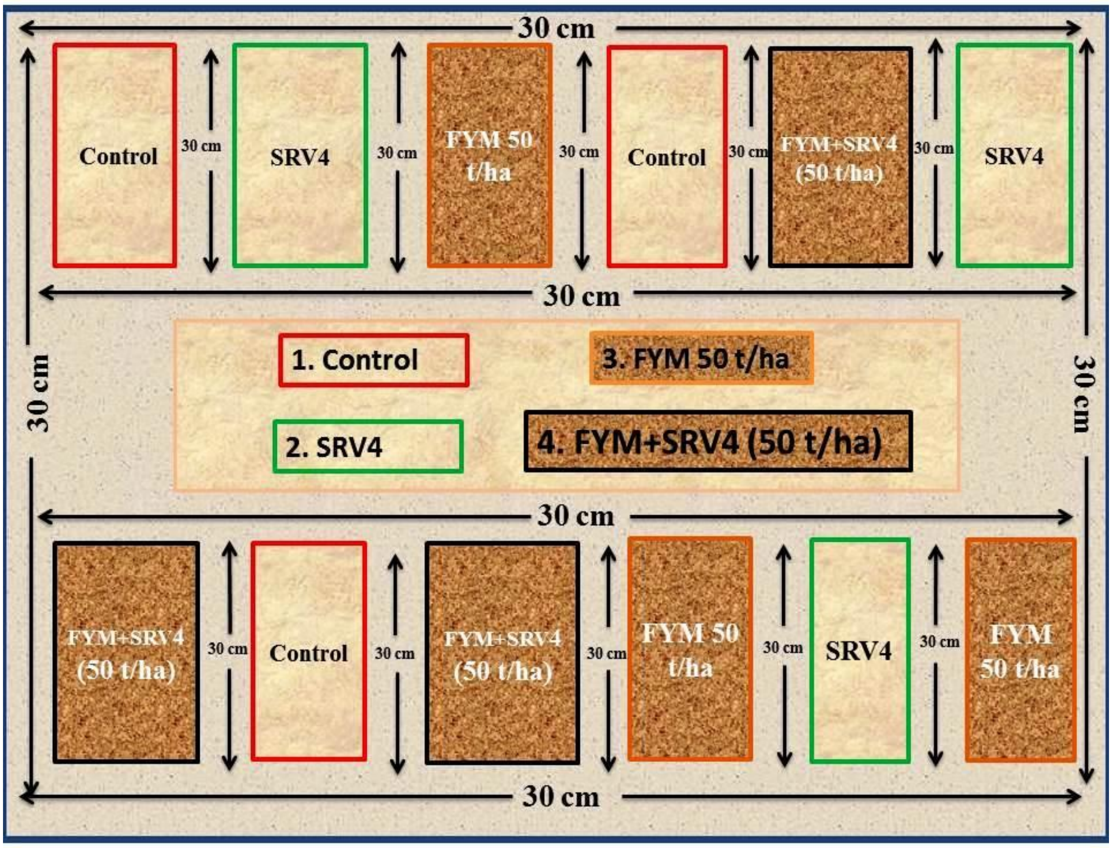

Figure 4.2 Experimental plots for the cultivation of paddy crop during 2015 and 2016

Figure 4.3 Experimental plots preparation for the transplantation of paddy seedlings **(A)** 2015 and **(B)** 2016

4.2.2 Application of Treatments

For both the study years (2015 and 2016), four treatments used in this experiment were:

1. Control
2. *C. albidum* SRV4 strain
3. FYM (50 t ha^{-1}) and
4. FYM (50 t ha^{-1}) + *C. albidum* SRV4 strain

4.2.3 Paddy Crop Cultivation in Field Condition

4.2.3.1 Paddy Nursery Establishment

For present investigation, the selected experimental crop was paddy (*Oryza sativa*). The rice variety namely HUR 3-4 (Hindu-University-Rice 3-4) was obtained from Department of Genetics and Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University (South campus), Mirzapur, Uttar Pradesh. Seeds sterilization and inoculum preparation was done as described in Chapter (3). The germinated seeds were transferred to plots for nursery development. The nursery of rice cultivar was prepared in month of June during both the years 2015 and 2016.

Figure 4.4 Paddy nursery establishments for the transplantation of paddy seedlings **(A)** 2015 and **(B)** 2016

4.2.3.2 Paddy Seedlings Treatment with Salt Tolerant SRV4 Strain

Before transplantation, the paddy seeding of equal height was removed from nursery plots. The roots of seeding was further dipped in SRV4 suspension (1×10^8) for 2 hours (Nandkumar et al., 2003) and finally transplanted in to the experimental plots.

Figure 4.5 Seedling treatments with isolated salt tolerant PGPR inoculant (*C. albidum* SRV4 strain) before transplantation during **(A)** 2015 and **(B)** 2016

4.2.3.2 Paddy Transplantation

Five paddy hills having equal height was transplanted in month for both the years 2015 and 2016 at 20cm×20cm dimensions in each plot. A total of 600 paddy hills were transplanted in each plot. The SRV4 inocula were sprayed on paddy plant parts on panicle initiation stage at the rate of 2 litres plot^{-1}.

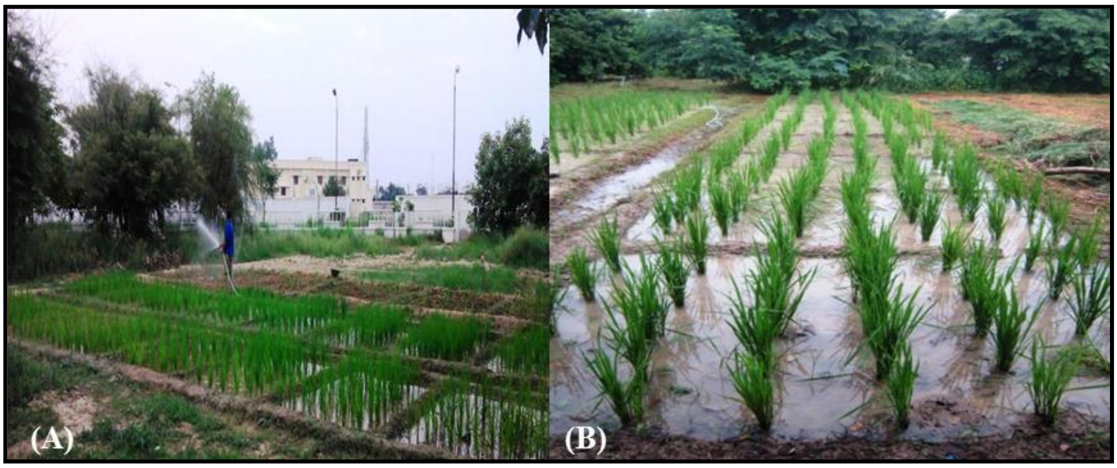

Figure 4.6 Paddy seedling transplantation during **(A)** 2015 and **(B)** 2016

4.2.3.3 Paddy Plant Growth Parameters and Antioxidant Enzymes Analyses

The paddy plant growth parameters (plant height, tiller and panicle numbers) and antioxidant enzymatic activities were monitored after panicle development stage during the paddy crop cycle in both the study years 2015 and 2016.

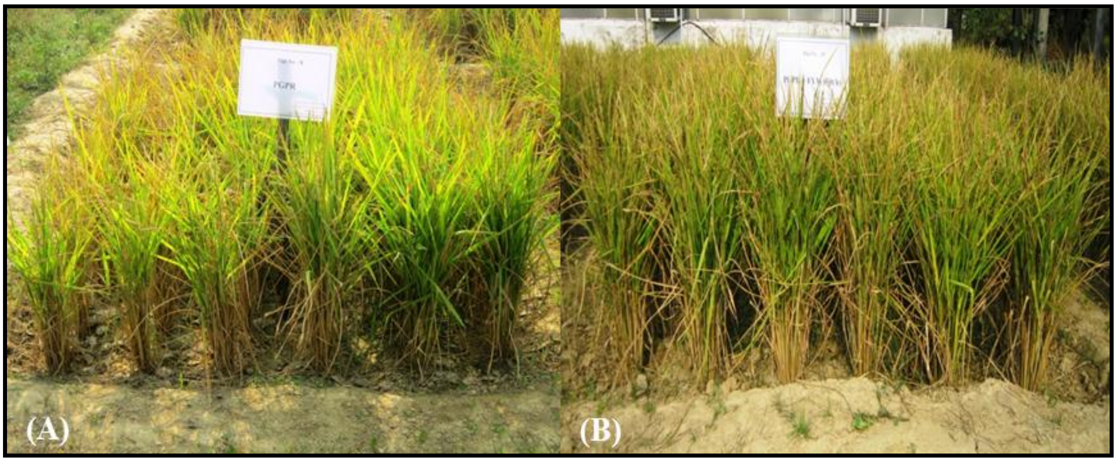

Figure 4.7 Paddy crop growth stage after panicle development during the crop cycle for study years **(A)** 2015 and **(B)** 2016

4.2.3.4 Paddy Crop Harvesting

The matured paddy crop was harvested during the month of November in both years 2015 and 2016. The rice grain yield parameters were measured after crop harvesting.

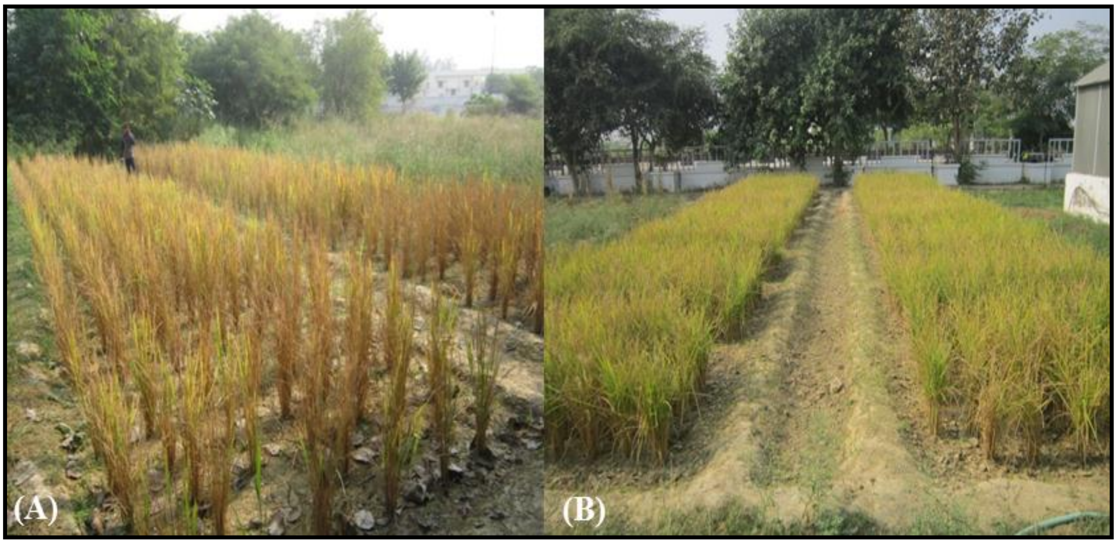

Figure 4.8 Matured paddy crop (A) 2015 and (B) 2016

CHAPTER 5

OBJECTIVE 1

TO ANALYZE THE IMPACT OF PGPR AND FARMYARD MANURE (FYM) APPLICATIONS ON SOIL PHYSICO-CHEMICAL PROPERTIES

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5.1 Introduction

Soil ecosystems surrounded by different climatic variables, is affected by variety of abiotic and biotic stresses. Natural and anthropogenic sources potentially affect the soil fertility and its functioning. Salt salinity is one of the principal edaphic features for declining crop productivity in salt affected soils throughout the world (Vimal et al., 2017). Salts in excess proportion disturbed the ionic equilibrium and causes oxidative stress (Nadeem et al., 2014; Kang et al., 2014). However in greater concentration salt diminish physical, chemical, microbiological soil microbial process and finally affects the plant growth. Enhancement in electrical conductivity due to soil salinity adversely affects the soil texture, bulk density, aeration capacity, permeability and essential nutrient contents (Tejada ad Gonzalez, 2005). Thus, sustainable management of agricultural fields affected by soil salinity a need of the hour for future crop production enhancement.

In the past few decades the research has reversed on their traditional solutions for protecting the most important natural resource soil. The first green revolution which reliance on hybrid varieties of crops for elevated crop yield in association of modern agricultural technologies, irrigation and heavy doses of chemical fertilizer harshly disturb the soils health and led to ecosystem disturbances. Uses of organic amendments such as mulch, manures, composts, biochars, etc. have been given serious attentions around the Globe for sustainable reduction and remediation of disturbed agro-ecosystems. Generally, the slightly acidic nature of composts/FYM has been reported effective in soil nutrient mineral solubilization, conductivity reduction and soil stabilization (Singh et al., 2010). Decline in Na^+ leaching, exchangeable sodium percentage and electrical conductivity are potentially associated with organic matter amendment (Singh et al., 2011). Several exogenous organic matters such as FYM and composts are effectively enhanced the soil organic carbon (SOC) levels (D'Hose et al., 2014; Vanden Nest et al., 2014). Andriamananjara et al. (2016) examined addition of FYM in paddy crop agriculture in highly weathered soils and concluded that FYM are effective in phosphorus (P) availability to plants. Thus, FYM may be considered as potential and possible amendments to restore and rejuvenate the soil productivity of stressed paddy agro-ecosystems.

The PGPR, an efficient group of microbes, can efficiently promote plant growth under abiotic and biotic stress environments. Several PGPR strains with their unique characteristics have been found to improve the productivity of *Oryza sativa* (Nautiyal et al., 2013), *Triticum aestivum* (Upadhayay and Singh, 2015) and *Lycopersicum esculantum* (Mayak et al., 2004; Tank and Saraf, 2010) under soil salinity. We also isolated and examined the use of *C. albidum* strain SRV4 under greenhouse pot experiment on paddy plant health under salinity stresses.

Since reports related to impact of FYM in combination with salt tolerant PGPRs application on soil physico-chemical characteristics in saline paddy filed are lacking therefore, this study was conducted to assess the impact of salt tolerant PGPR *C. albidum* SRV4 strain + FYM on soil properties under field conditions. We hypothesize that FYM in combination with salt tolerant *C. albidum* SRV4 strain will be effective in improving soil conditions and soil salinity amelioration of paddy fields. The improved soil conditions due to FYM and plant growth promoting attributes of *C. albidum* SRV4 strain would be beneficial to remove the stresses because of salinity and ultimately a better soil health and its functioning.

5.2 Materials and Methods

5.2.1 Soil Sampling and Analysis of Soil Physico-Chemical Properties

From each experimental plot soil samples were collected in month of October during paddy crop cycles in 2015 and 2016. Monoliths of soils of size (10cm×10cm×10cm) were randomly sampled from each experimental plot (Singh et al., 2010; 2013). The soil samples were immediately transported to laboratory, spread over the paper sheet for air drying before the analysis. Subsequently, the air dried soil samples were passed through the sieve (mesh size 2mm) and stored in polythene bags at 4°C for subsequent analysis. The relevant physicochemical parameters are given below.

5.2.2.1 Soil pH

The soil pH was examined according to Thomas (1996). About 10 g of soil samples were dissolved in 25 mL of distilled water and mixed with the glass rod. The pH was examined with the pH meter in triplicate.

Figure 5.1 Soil pH measurements

5.2.2.2 Electrical Conductivity

The soil EC was examined according to Rhoades (1982). The soil was dissolved in (1: 2.5, soil: water) water and reading was measure in triplicate with the EC meter.

Figure 5.2 Soils electrical conductivity measurements

5.2.2.3 Bulk Density

Bulk density of collected samples was determined by the method of Donahue et al. (1983). In brief:

i. The soils sample was collected with soil core.
ii. The soil with soil core was dried at 105 °C for 12 h in an oven and weight was taken.
iii. The exact volume of soil was determined by measuring the cylinder core volume.

Bulk density = weight of oven dried soil (gm)/ volume of soil core (cm^3)

5.2.2.4 Total Soluble Salts (TSS)

Total soluble salts refers to the total amount of soluble salts in soil saturated paste extract expressed in parts per million of mg L^{-1}. A linear relationship exists between TSS and EC in certain range. TSS was calculated from EC according to Maithi, 2003 with formula given below.

Total soluble salts (mg L^{-1}) = Electrical conductivity (mMhos/cm) ×640

5.2.2.5 Organic-C

Organic-C in soil samples was estimated by rapid dichromate oxidation method as described by Walkley and Black (1934). The steps are:

i. About 0.50 gm of soil samples (passed through 0.2 mm sieve) was taken in 500 mL conical flask.
ii. Added 10 mL of 1N $K_2Cr_2O_7$ in flask and gently swirled. The flask was kept on asbestos sheet.
iii. Added 250 mL of conc. H_2SO_4 (containing 1.25 % $AgSO_4$) very carefully from a measuring cylinder. Gently swirled few times. Kept this flask for 30 minutes and protects from droughts.
iv. Added 20 mL of DW and 10 mL of orthophosphoric acid to get the end point of titration.
v. Added 1 mL of diphenylamine indicator and titrated with ferrous ammonium sulphate till the colour flashes from blue violet to green. Similarly control was run without soil.
vi. The data was computed as formula given below.

Organic-C (%) = 10(B-T)/B×0.003×100/S

Where

B= Volume of ferrous ammonium sulphate required for blank titration in mL

T= Volume of ferrous ammonium sulphate required for soil sample in mL

S = Weight of soil in g

5.2.2.6 Available-N

Available-N was estimated by potassium permanganate method by Subbaih and Ashija, 1956. The detailed steps are as given below.

i. Taken 20 gm of soil sample in 800 mL Kjeldal flask, added 20 mL of water, 100 mL of 0.32% $KMNO_4$, and 100 mL of 2.5% NaOH solution.
ii. The excess frothing during heating was prevented by adding few glass beads.
iii. The digested product was collected in a conical flask containing 20 mL boric acid and indicator solution.
iv. With the absorption of NH_3 gas the pinkish colour boric acid solution turns to green.
v. Nearly 100 mL of distillate was collected and titrated with 0.02 N H_2SO_4.
vi. The blank correction (without soil) was made for final calculation.

Available N (ppm) = $(A-B)\times N\times 14\times 10^3/W$

Available N (%) = Nitrogen in ppm/ 10,000

Available N (Kg/ha) = N (%) × 22500

Where

A= Volume of H_2SO_4 consumed for blank in mL

B= Volume of H_2SO_4 consumed for soil sample in mL

N = Normality of H_2SO_4

W= Weight of soils sample

5.2.2.7 Available-P

Available phosphorus in soil was estimated by Olsen method (Olsen et al., 1954). The steps are given below.

i. Added 2.5 gm of soil sample in 50 mL of extracting solution ($NaHCO_3$) in 250 mL conical flask.
ii. Flask shaken with magnetic shaker and filter suspension with Whatman filter paper No-4.
iii. Added activated Carbon (free of P) to obtain clear filtrate.
iv. Shake flask immediately before pouring the suspension into the funnel.
v. Taken the 5 mL of extract into 25 mL volumetric flask and added 5 mL of Dickman and Brays reagent drop by drop with constant shaking till the effervescence stopped.
vi. The neck of the flask was washed and contents were diluted to about 22 mL (If pH is less than 5.0 then acidify with 5 NH_2SO_4 to pH 5).
vii. Then, 1 mL of diluted $SnCl_2$ was added and volume was made up to the mark.
viii. The colour was stable for 24 h and maximum intensity was obtained in about 10 minutes.
ix. The data was calculated with formula below.

Olsen P (Kg/ha) = $R \times V/v \times 1/S \times 2.24 \times 10^6/10^6$

Where

V = Total volume of extract

v = Volume of aliquot taken for analysis

S = Weight of soil

R = Weight of P in the aliquot in µg (from standard curve)

5.2.2.8 Available-K

Available-P of soil samples was estimated by flame photometer (Systronics-130) according to method of Jackson (1973).

5.2.3 Statistical Analyses

The data are expressed as mean of three replicates ± SE for both the years 2015 and 2016. The ANOVA was performed to test the significance of the observed differences using SPSS (Version 20: IBM, Armonk, NY, USA). Data were compared with Duncan's multiple range test (DMRT) at $p<0.05$.

5.3 Results

The results of different FYM+SRV4 amendments on soils physico-chemical properties are shown in Table 5.1. ANOVA indicated significant variations ($P= < 0.01$ to < 0.001) in soil physico-chemical properties due to various treatments (Table 5.1). The pH, EC, BD and TSS was maximum in the control plots and minimum in FYM+SRV4 amended plots in both years. The pH was observed maximum (8.66±0.14) in control plots and minimum (7.22±0.08) in FYM+SRV4 amended plots.

Table 5.1 Variation in soil physico-chemical properties in 2015 and 2016 crop cycle. Values are means of three replicates ±SE. The superscript letter denote significant difference between means at $p<0.05$.

Soil parameters	**Year**	**Treatments**				**F-value (For each year N=12)**
		Control	**SRV4**	**FYM**	**FYM+SRV4**	
pH	2015	8.66 ± 0.14^{a}	8.26 ± 0.10^{b}	7.86 ± 0.16^{c}	7.40 ± 0.13^{d}	15.251*
	2016	8.52 ± 0.85^{a}	8.08 ± 0.10^{b}	7.70 ± 0.11^{c}	7.22 ± 0.08^{d}	31.784*
EC (dS m^{-1})	2015	6.97 ± 0.13^{a}	6.46 ± 0.18^{b}	5.45 ± 0.17^{c}	4.68 ± 0.16^{d}	38.645*
	2016	6.48 ± 0.13^{a}	6.20 ± 0.12^{a}	5.10 ± 0.11^{b}	4.48 ± 0.12^{c}	55.116*
BD (g cm^{-3})	2015	1.55 ± 0.02^{a}	1.51 ± 0.03^{a}	1.48 ± 0.02^{ab}	1.41 ± 0.03^{b}	3.637*
	2016	1.52 ± 0.02^{a}	1.49 ± 0.02^{ab}	1.44 ± 0.02^{bc}	1.40 ± 0.03^{d}	4.371**
TSS (mg L^{-1})	2015	4464 ± 88.12^{a}	4138 ± 116.9^{b}	3493 ± 109.5^{c}	2997 ± 105.3^{d}	38.645*
	2016	4149 ± 88.37^{a}	3968 ± 79.56^{a}	3269 ± 72.82^{b}	2869 ± 81.04^{c}	55.116*
Organic-C (%)	2015	0.56 ± 0.024^{c}	0.68 ± 0.034^{c}	0.90 ± 0.042^{b}	1.66 ± 0.070^{a}	112.77*
	2016	0.60 ± 0.028^{c}	0.71 ± 0.026^{c}	0.97 ± 0.055^{b}	1.75 ± 0.061^{a}	125.96*
Available -N (Kg ha^{-1})	2015	108 ± 3.69^{c}	120 ± 4.22^{c}	133 ± 4.10^{b}	160 ± 7.02^{a}	20.388*
	2016	111 ± 4.30^{c}	129 ± 4.01^{b}	138 ± 3.91^{b}	165 ± 6.57^{a}	21.525*
Available-P (Kg ha^{-1})	2015	17.07 ± 0.55^{c}	18.94 ± 0.76^{c}	21.70 ± 0.85^{b}	25.31 ± 1.29^{a}	15.521*
	2016	17.70 ± 0.84^{c}	20.27 ± 0.94^{bc}	22.97 ± 1.19^{b}	27.55 ± 1.42^{a}	13.988*
Available-K (Kg ha^{-1})	2015	139 ± 3.11^{c}	142 ± 3.58^{c}	165 ± 4.43^{b}	177 ± 5.05^{a}	20.252*
	2016	140 ± 3.19^{c}	147 ± 2.68^{c}	168 ± 4.33^{b}	179 ± 4.82^{a}	22.306*

F: variance ratio; *Significant value **$P <0.01$,*$P < 0.001$

Similarly, the EC was recorded maximum (6.97±0.13) in control plots and minimum (4.48±0.12) in FYM+SRV4 amended plots. The BD was observed highest (1.55±0.02) and lowest (1.40±0.03) in FYM+SRV4 amended plots. The soil TSS was maximum (4464±88.12) in control plots and minimum (2869±81.04) in FYM+SRV4 amended plots. However, the Organic-C, available-N, available-P, and available-K was maximum in the FYM and SRV4 treated plots and minimum in control plots in the both years. Organic-C was found minimum (0.60±0.028) in control plots while maximum (1.75±0.061) was found in FYM+SRV4 amended plots. Available-N was recorded minimum (108±3.69) in control plots and maximum (165±6.57) in FYM+SRV4 treated plots. Available-P was observed lowest (17.07±0.55) in control and greatest (27.55±1.42) in FYM+SRV4 amended plots. Similarly, the available-K was also found minimum (139±3.11) in control plots and maximum (179±4.82) in FYM +SRV4 amended plots.

5.4 Discussion

In soil salinity affected region, the low soil nutrient availability due to soil disturbances and higher salt concentrations affects soil fertility, agriculturally important microbial community composition and paddy crop productivity. The stress soil conditions and lower nutrient contents may affects the microbial mediated nutrient cycling processes responsible for nutrient supply to paddy crops (Singh et al., 2010). The soils of the present experimental sites are nutrient poor and salinity affected, therefore, organic amendments like FYM in combination with suitable salt tolerant microbial inoculants addition could be very crucial to improve he soil conditions and consequently the crop productivity. In previous investigations for the purpose of obtaining the maximum paddy crop yields importance has been given to the use of fly ash (FA) and FYM to restore and conserve the soil moisture and improve soil nutrient status of nutrient poor agriculture soils (Pandey and Singh, 2010).

A statistically significant variations in soil physico-chemical properties such as pH, BD, EC, TSS, organic-C, available-N, etc. was noted for year 2015 and 2016 (Table 5.1) after FYM and SRV4 strain treatments. The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant reduction in soil pH, EC, TSS and BD. These reductions were maximum in FYM+SRV4 treated plots, indicating that impact of FYM is potential in minimizing the soil salinity impact, which is not surprising because other scientific reports also demonstrated that addition of FYM in conjunction with other amendments improves the physico-chemical and biological properties of soils (Jala and Goel, 2006; Lee et al., 2006). Acidic nature of FYM are effectively balanced the alkaline nature of soils due to managing Acid-base equilibriums. In paddy crop cycle we observe that the FYM amendment plots efficiently declined electrical conductivity and thus efficiently reduced soil salinity. Application of FYM in agriculture was found to change the chemical properties of soil, and the most important effect was to increase soil pH (Wong and Wong, 1990). Singh et al. (2010) demonstrated that the application of FYM with pyrite greatly improved the soil properties in a rain fed saline paddy agro-ecosystem (Singh et al., 2010). Decline in soil compactness due to FYM amendment favours reduction in soil BD. Declined BD supports effective aeration capacity, high water availability to roots and supports healthy root architecture. In the present experiment, the drastic increase in soil organic-C in FYM amended plot compared to control (Table 5.1) might be due to the alteration in the organic composition of soil because of FYM contains rich amount of decay and decomposed organic materials. High organic-C is beneficial to create low BD, aggregate stability, high porosity and improved soil structure, all of which lead to increased water holding capacity and plant available water (Murphy, 2014). In present study, compared to other treatments, available-N, -P and -K was greatly increased in FYM+SRV4 treated plots in both the study years 2015 and 2016 (Table 5.1) could be due to presence of plenty amount of inorganic nutrients present in FYM (Singh et al., 2010; 2013). Further, the SRV4 strain may efficiently solubilize the organic matter into inorganic available nutrients like N, P and K (Halverson et al., 2000; Torres et al., 2015) and consequently, a greater amount of available-N, -P and -K might be expected in FYM and SRV4 treated plots compared to control one. The FYM is a good source of K content as observed in a study by Abe et al. (2016).

5.5 Conclusions

This field experiment highlights the beneficial tripartite soil-microbe-manure interaction under saline paddy crop agriculture. Application of organic FYM amendments with efficient PGPR SRV4 strain will definitely be an excellent opportunity for soil salinity removal and fertility restoration. The salt tolerant SRV4 bacterium in combination with FYM effectively stabilizes the organic matter content and improves the availability of available N, P and K soils nutrients in saline soils. The results showed improvement in soil qualities and decline in soil pH due to FYM+*C. albidum* SRV4 strain amendment and therefore, diminish the salt toxicity in two consecutive years 2015 and 2016. In conclusion, FYM with SRV4 microbial bio-inoculant amendment was effective in restoration of saline agriculture and *C. albidum* SRV4 strain will be novel agriculturally beneficial microbial agent for sustainable paddy crop production in salinity affected area.

CHAPTER 6

OBJECTIVE 2

TO EXAMINE THE IMPACT OF PGPR AND FYM APPLICATIONS ON PLANT GROWTH PARAMETERS AND ANTIOXIDANT LEVEL IN PADDY PLANT IN SALINE SOIL

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6.1 Introduction

The rice (*Oryza sativa* L.) has been declared as the vital staple food for billions of population, and provides nutritional food to one-half of the world's people (Nakbanponte et al., 2014). Consequences of indiscriminate human population rise in coming years; about 536-551 million tons of Global rice demand is expected. The paddy, one of the highly consumed cereals, is chiefly cultivated by developing countries (FAO, 2012). However, during paddy cultivation in plane or flooded regions, the water used during irrigation to the paddy soil, is either used by crop plants or percolates downward or evaporates directly to the atmosphere. After water evaporation, huge amount of salt accumulates on the soil surface of paddy fields. In flooded paddy agro-ecosystems, the soil salinity could adversely affect paddy plant nutrient uptake; affect beneficial microbial community compositions, soil fertility and ultimately the paddy crop yields and. Therefore, the paddy crop may be considered as the excellent experimental crop for the study of its physiological/biochemical parameters under salinity stresses (Khan and Hemlata, 2016).

Soil salinization of productive agriculture lands, principally with salt (NaCl), is most persistent and detrimental to paddy crop productivity. High salt concentrations accumulate both Na^+ and Cl^- simultaneously, although the effects of these two ions may vary (Nakbanponte et al., 2014). Na^+ accumulation declines soil porosity, soil aeration and water conductance (Porcel et al., 2012). High Na^+ ions also interfere with

K^+ and Ca^{2+}; affect stomatal regulation, photosynthesis and enzymatic activities (Paul and Lade, 2014). Salinity diminishes a number of paddy plant growth parameters including panicles initiation, tiller number, spikelet formation, delayed heading and grain size (Nakbanponte et al., 2014). Deterioration of soil physico-chemical conditions due to salinity thus hinders crop plant nutrient uptake from soils, and ultimately, its productivity (Paul and Lade, 2014).

Plant-microbe interactions play vital roles in the maintenance of plant and soil productivity under stress environment (Singh 2015; Vimal et al., 2017). Stressed soil conditions adversely influence the microbial community composition and their population size in agriculture soils (Vimal et al., 2017). Under such soil conditions, plants construct favourable rhizospheric conditions by recruiting and attracting the beneficial microbial communities by secretion of root exudates- sugars, amino acids, organic acids and signalling molecules (Singh, 2015; Tiwari and Singh, 2017). The favourable rhizospheric conditions may harbour enough beneficial microbial agents and in turn, the microbes provides several plant growth promoting benefits to crop plants (Egamberdieva et al., 2008; Mendes et al., 2011). Plant growth promoting rhizobacteria (PGPR) in close association with the root zones of saline soils, invite strong attention and attracts researchers owing to their versatility, survival and unmatchable plant beneficiary attributes under harsh soil conditions (Cardinale et al., 2015; Vimal et al., 2016). The PGPR promote plant growth through various mechanisms such as bio-film formation (Petrova and Sauer, 2016), phytohormones production (Barnawal et al., 2014), nutrients management (Singh et al., 2011) and antioxidants production (Upadhayay et al., 2012). However, the unknown mechanisms performed by PGPR under different stress environmental conditions such as soil salinity (Barnawal et al., 2014), drought (Mayak et al., 2004; Sandhya et al., 2009), metal contamination (Grobelak et al., 2015) and biotic stresses (Barnwal et al., 2017), are also reported in favour of crop health. Inoculation of PGPR directly to soils or bio-priming of seeds and seedlings with the PGPR, has been found to enhance seed germination and viability of seedlings in salt affected soils (Mayak et al., 2004; Nautiyal et al., 2013).

To survive various abiotic and biotic stresses, plants have evolved well developed antioxidative systems (Gill and Tuteja, 2010). Potential PGPR strains are also reported in the management of stresses by harmful radicals produced by plants during metabolism (Upadhay et al., 2012). Antioxidants and antioxidative enzymes in plants, inoculated with PGP microbial agents, have been reported to scavenge the harmful radicals and minimize the stress induced damages (Khan et al., 2016). Antioxidants not only act as the compatible osmoprotectants but also serve as signalling molecules to modulate the osmotic balances of cell cytoplasm, and trigger the expression of specific genes, essential for the wellbeing of plant physiology and metabolism (Cui et al., 2016). It appears that PGPR could be an important group of beneficial microbes that can be exploited to alleviate the abiotic and biotic stresses in crop plants. Therefore, addition of salt tolerant microbial inoculants to the soil could be the novel and cost-effective alternative to alleviate salinity stress in paddy crops. The isolation and identification of new salt tolerant PGPRs strains can be exploited to develop effective and viable bio-inoculants to enhance paddy production in salinity affected areas. There are scanty reports on application of PGPR inoculants to alleviate salinity stress, particularly the role of *Curtobacterium* sp. on paddy crop. Hence, the present study was conducted to investigate the efficacy of salt tolerant PGPR strains isolated from saline soils on paddy plant growth promotion under salt stresses. The most efficient isolate *C. albidum* SRV4 was selected on the basis of its *in-vitro* PGP activity and 16S r-RNA gene sequencing for application. A greenhouse experiment was conducted to examine the effect of SRV4 inoculation on plant growth parameters and antioxidant activities of paddy plants under salinity.

6.2 Material and Methods

6.2.1 Paddy Plant Growth Parameters and Rice Grain Yield

6.2.1.1 Plant Height

The plant samples having longest leaves was selected and sampled on November 11, 2015 and November 13, 2016. The measurements of sample paddy plant height were done with a meter scale as described by to Singh et al. (2010).

6.2.1.2 Tiller Number

The paddy plant tiller numbers were counted according to Singh et al. (2011).

6.2.1.3 Panicle Number

The total panicle number/meter square of paddy plots was counted according to Fageria et al. (2009).

6.2.1.4 Weight of 1000 Rice Grains

The weight of 1000 rice grains was measured according to Fageria et al. (2014) with the standard weighing methods.

6.2.1.5 Rice Grain Yields

The paddy plant growth and yield components as number of panicle or head per unit area, grain per panicle, % of filled grains, and weight of thousand grains was separately calculated. The final rice grain yield was computed at a rate of per hectare according to Fageria et al. (2014) with the formula given below.

$$\text{Grain yield (t ha}^{-1}\text{)} = \text{number of panicles m}^{-2} \times \text{number of grains per panicle} \times \text{\% of filled grains} \times \text{weight of 1000 grain (g)} \times 10^{-5}$$

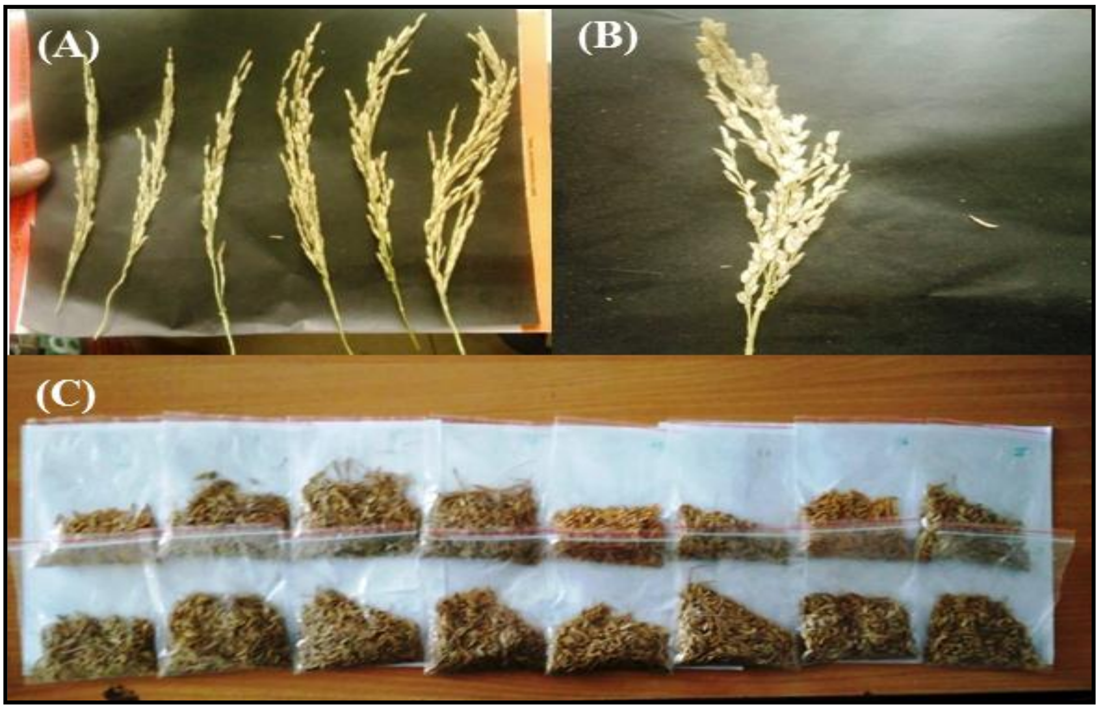

Fig 6.1 Crop yield components **(A**) Panicles **(B)** Panicle length **(C)** 1000 grain weight

6.2.2 Antioxidants Enzymatic Properties

For measurement of antioxidative enzymatic properties, the fresh paddy plant green leaves were sampled on October 25, 2015 and October 26, 2016. Fresh leaf (about 0.2 gm) was homogenised in 2 mL of 50 mM ice cold phosphate buffer (pH 7.8) containing 1 mM EDTA with sterilized mortar and pestle. The homogenate was centrifuged at 10,000×g for 15 min at 4 °C. The supernatant was used to assess the enzyme at 4 °C (Wang et al., 2012). The specific enzyme activity for all enzymes was expressed as unit mg^{-1} protein.

Catalase (EC 1.11.1.6) activity was examined through observing the decomposition of H_2O_2 at 240 nm (Aebi, 1983). The reaction mixture (3 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 0.1% H_2O_2, and 0.1 mL of enzyme extract. The reaction was commenced through addition of enzyme extracts. The decrease in H_2O_2 levels was determined by computing the absorbance at 240 nm with spectrophotometer and quantified using extinction coefficient (36 mM^{-1} cm^{-1}).

Superoxide dismutase (EC 1.15.1.1) activity was executed by measuring its effectiveness in inhibiting the photo reduction of nitro blue tetrazolium (NBT) as described by Giannopolitis and Ries (1977). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 0.75 mM NBT, 0.02 mM riboflavin and 0.1 mL enzyme extract. Riboflavin was added as the last component and the reaction was initiated by placing the tubes under two 20 W fluorescent lamps, which lasted for 10 min. Non-illuminated and illuminated reactions without enzyme extract served as calibration standards. The absorbance values of the reaction mixture and the blank control were measured at 560 nm with a spectrophotometer. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate, and the results were expressed as unit mg^{-1} protein of FW. One unit of SOD was defined as the amount of enzyme that inhibits 50% NBT photo-reduction.

Peroxidase (EC 1.11.1.7) activity was based on oxidation of guaiacol using hydrogen peroxide (Zhang et al., 1996). The reaction was initiated by adding 20 µL of the enzyme extract to 3 mL of reaction mixture consisting of 100 mM phosphate buffer (pH 7.0), 20 µL of guaiacol solution, and 10 µL of hydrogen peroxide solution. The absorbance was measured at 470 nm at time points of reaction initiation and 5 min later with spectrophotometer. Enzyme activity was quantified based on the amount of tetraguaiacol formed using the extinction coefficient (26.6 mM^{-1} cm^{-1}). A unit of peroxidase activity was expressed as µmol mL^{-1} H_2O_2 decomposed per minute.

Ascorbate peroxidase (EC 1.11.1.11) activity of was assayed according to Nakano and Asada (1981). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1mM EDTA, 0.5mM ascorbate, 0.1mM H_2O_2 and 0.1 mL enzyme extract. The reaction was initiated by addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 3 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}). One unit of APX was defined as 1 mmol mL^{-1} ascorbate oxidized per minute.

6.3 Statistical Analyses

The data are expressed as mean of three replicates ± SE for both the years 2015 and 2016. The ANOVA was performed to test the significance of the observed differences using SPSS (Version 20: IBM, Armonk, NY, USA). Data were compared with Duncan’s multiple range test (DMRT) at $p<0.05$

6.4 Results

6.4.1 Effect of FYM+SRV4 Amendments on Paddy Plant Growth Parameters and Rice Grain Yields

Paddy plant growth variables (plant height, tiller number, panicle number) and rice grain yields was noted maximum in SRV4+FYM amended plots compared to other treatments (Table 6.1). ANOVA showed significant variations in paddy plant growth variables ($P < 0.001$) and rice grain yields ($P= < 0.01$ to < 0.001) due to treatments in both the years 2015 and 2016 (Table 6.1). Two-way ANOVA for the pooled data of paddy plant growth variables and rice grain yield across different treatments and years showed that impact of treatment was only significant ($P < 0.001$), while impact of years and treatment × year interaction was not significant (Table 6.3). Across different treatments maximum paddy plant height in both the years 2015 (135±6.77 cm) and 2016 (139±7.68 cm) was noted in FYM+SRV4 amended plots compared to other treatments. The maximum tillers per plant were appears in FYM+SRV4 amended plots in both crop cultivation years. Similarly the panicles were recorded maximum in FYM+SRV4 amended plots in 2015 and 2016. For both the years, also the weight of 1000 rice grain was found maximum in FYM+SRV4 amended plots compared to other treatments. The maximum rice grain production was found in FYM+SRV4 amended plots for 2015 (5.04±0.53 t ha^{-1}) and 2016 (5.85±0.57 t ha^{-1}).

Table 6.1 Variation in paddy plant growth variables and rice grain yields during paddy crop cycle 2015 and 2016.Values are means of three replicates ±SE. the superscript letter denote significant difference between means at $p<0.05$.

Agronomic variables	**Year**	**Treatments**				**F-value (For each year N=12)**
		Control	**SRV4**	**FYM**	**FYM +SRV4**	
Plant height (cm)	2015	87±2.92^{c}	112.15±7.55^{b}	110.71±6.01^{b}	135±6.77^{a}	10.415**
	2016	83±7.01^{b}	120.00±10.21^{a}	115.00±5.01^{a}	139±7.68^{a}	9.115**
Tiller number (Plant^{-1})	2015	4.00±0.57^{c}	5.33±0.33^{bc}	6.00±0.57^{b}	8.00±0.57^{a}	10.00**
	2016	4.00±0.57^{c}	5.66±0.33^{b}	6.00±0.57^{bc}	8.00±0.57^{a}	9.70**
Panicle number (m^{2})	2015	236±16.25^{c}	260±10.40^{bc}	289±7.81^{ab}	321±13.07^{a}	8.92**
	2016	245±17.38^{c}	286±13.31^{bc}	300±11.54^{ab}	335±13.22^{a}	6.93**
Weight of 1000 rice grains (g)	2015	12.72±1.00^{b}	15.03±1.15^{b}	16.57±0.75^{b}	23.99±1.85^{a}	14.89*
	2016	12.80±1.08^{c}	18.05±1.72^{bc}	19.50±2.56^{ab}	25.12±1.80^{a}	7.34**
Rice grain yields (t ha^{-1})	2015	1.53±0.18^{c}	2.09±0.20^{bc}	2.74±0.11^{b}	5.04±0.53^{a}	25.23*
	2016	1.78±0.26^{b}	2.96±0.67^{b}	3.26±0.78^{b}	5.85±0.57^{a}	8.037**

F: variance ratio; **$P <0.01$,*$P < 0.001$; NS = Not Significant

6.4.2 Effect of FYM+SRV4 amendments on Antioxidant Enzyme Activities

Antioxidant enzyme activities (CAT, SOD, POX and APX) were found lowest in SRV4+FYM amended plots and highest in control plots (Table 6.2). ANOVA showed significant variations ($P= < 0.01$ to < 0.001) in antioxidant enzyme activities in both the years 2015 and 2016 during paddy crop cycles (Table 6.3). When the data of antioxidant enzyme activities were polled across different treatments and years, two-way ANOVA showed significant differences ($P < 0.001$) only for treatments, while impact of years and treatment × year interaction was not significant (Table 6.3).

Table 6.2 Variation in anti-oxidative enzyme activities during paddy crop cycle 2015 and 2016.Values are means of three replicates ±SE. the superscript letter denote significant difference between means at $p<0.05$.

Antioxidant Enzymes	Year	Treatments				F-value (For each year N=12)
		Control	SRV4	FYM	FYM +SRV4	
CAT (Unit mg^{-1} Protein)	2015	14.90±1.12^{a}	12.78±0.68^{ab}	13.41±0.48^{a}	10.92±0.73^{b}	6.898**
	2016	14.93±0.52^{a}	12.95±0.84^{a}	13.01±0.62^{a}	10.27±0.60^{b}	8.385**
SOD (Unit mg^{-1} Protein)	2015	2.46±0.20^{c}	2.06±0.07^{b}	2.10±0.05^{bc}	1.60±0.04^{a}	9.175**
	2016	2.28±0.11^{a}	1.97±0.12^{a}	2.03±0.06^{a}	1.56±0.09^{b}	8.664**
POX (Unit mg^{-1} Protein)	2015	45.09±1.38^{a}	41.06±1.32^{a}	41.93±1.27^{a}	35.83±1.35^{b}	8.274**
	2016	44.19±1.40^{a}	39.98±1.54^{a}	40.15±1.54^{a}	32.04±1.21^{b}	12.587*
APX (Unit mg^{-1} Protein)	2015	45.82±1.62^{a}	39.29±1.99^{a}	40.02±2.57^{a}	30.96±1.92^{b}	8.834**
	2016	42.28±1.76^{a}	36.75±1.22^{b}	38.12±1.41^{ab}	28.43±1.36^{c}	15.902*

F: variance ratio; **$P<0.01$,*$P<0.001$

6.5 Discussion

In this chapter, impact of isolated salt tolerant PGPR *C. albidium* SRV4 strain and FYM applications on paddy plant growth parameters and antioxidant levels has been discussed. The efficient salt tolerant PGPR SRV4 strain with FYM amendments (50 t ha^{-1}) nourishes degraded soils, enhances availability of nutrients, modulates plant antioxidants and improves plant health and crop yield. Enhanced grain number in panicle and grain weight positively correlated with higher grain yield. Plant structures (Plant height, Tillers, Panicle) evidently determine SRV4 bacterium potentiality in both field trials. Reactive oxygen species (ROS) was excessively generated as a by-product of saline stress, trigger hypersensitive cell death (HR) due to ionic disorder in paddy plants. In order to fight back the toxic effect of high level of ROS, paddy plants are bestowed with large panel of antioxidant enzymes (Singh et al., 2013). The SRV4 strain amended with FYM significantly reduced electrical conductivity of soils. Thus reduce salinity in soils. Reduced salinity in soils positively reduced antioxidant activity in soils. Under saline conditions excess ROS require excess antioxidants to maintain the equilibrium for proper plant health. In this study the activity of antioxidant enzymes CAT, SOD, POX and APX was significantly reduced in paddy plants treated with FYM+SRV4 amendments, indicating that the PGPR SRV4 strain efficiently reduces the stress of paddy plants. Study of Upadhyay et al. (2012) also showed that in wheat leaves, the *Bacillus subtilis* and *Arthrobacter* sp. treatments effectively reduced CAT, APX and GR activity. Kang et al. (2014) observed *Pseudomonas putida* significantly decline the SOD and DPPH-radical scavenging activity in soybean plants suffering with oxidative stress. Recently Balseiro-Romero, (2017) with other workers observed that *Arthrobacter* sp., *Acinetobacter oleivorans* and *A. calcoaceticus* significant reduces CAT, SOD, GR, APOD and GPOD activities in *Cystisus striatus* and *Lupis lutens* plants in contaminated soils.

As the data of present study indicated that the antioxidative enzymatic activities such as CAT, SOD, POX and APX increased in paddy plants in control (without FYM or SRV4 strains) plots. It may be argued that the paddy plants may have developed defense enzymatic systems by synthesizing sufficient amount of antioxidants to alleviate and re-nature the damages caused by salinity mediated oxidative stress (Upadhyay et al., 2012). In general, the increase in the antioxidative enzyme activities in paddy plants in response to stress conditions i.e. un-treated soil of paddy fields may be directly correlated with the levels of stress substance accumulation in the leaves of paddy plants (Singh and Pandey, 2013). In the present experiments, an enhanced antioxidative enzymes production in paddy plants of control plots (Table 6.2), possibly could be to neutralize the adverse impact of higher salinity stress and is in conformity with the results of Gill and Tuteja (2010) who demonstrated higher antioxidant ability of plants under abiotic stress.

Table 6.3 Two-way ANOVA to examine the impacts of treatments, years and treatments × year interaction on paddy plant growth parameters and antioxidant activities. This analysis was performed on pooled data (mean values) across different treatments and years.

Source	**Treatments (DF=3) F-value**	**Years (DF=1) F-value**	**Treatments × Years (DF=3) F-value**
Plant height (cm)	18.96*	0.383^{NS}	0.260^{NS}
Tiller number (Plant^{-1})	19.65*	0.050^{NS}	0.050^{NS}
Panicle number (m^{2})	15.43*	2.64^{NS}	0.159^{NS}
Weight of 1000 rice grains (gm)	19.01*	2.52^{NS}	0.407^{NS}
Rice grain yields (t ha^{-1})	22.91*	3.17^{NS}	0.166^{NS}
CAT (Unit mg^{-1} Protein)	15.18*	0.220^{NS}	0.169^{NS}
SOD (Unit mg^{-1} Protein)	17.75*	1.45^{NS}	0.153^{NS}
POX (Unit mg^{-1} Protein)	20.70*	3.71^{NS}	0.458^{NS}
APX (Unit mg^{-1} Protein)	22.31*	4.34^{NS}	0.072^{NS}

DF: degree of freedom; F: variance ratio; *P < 0.001; NS = Not Significant.

6.6 Conclusions

Based on the above results, this study demonstrated that inoculation of *C. albidum* SRV4 had significant impact on paddy growth due to their plant growth promoting attribute under soil salinity stress. Thus it is evident from the data generate during this field experiments that *C. albidum* strain SRV4 has the potential to effectively mitigate negative impacts of soil salinity on paddy crop plants. In association with farmyard manure (FYM), the SRV4 strain positively enhances the paddy plant growth parameters due to declining the salinity stress and antioxidant enzymatic levels. The SRV4 strain with FYM as soil conditioner is effective in management of soil salinity problems of paddy agro-ecosystem. The results of present experiment showed that isolated salt tolerant SRV4 strain as microbial inoculant showed good performance with FYM (50 t ha^{-1}) in enhancement of paddy crop performance and rice grain in saline affected area.

CHAPTER 7

OBJECTIVE 3

TO ASSESS THE CORRELATION BETWEEN THE PGPR AND FYM APPLICATION WITH PADDY PLANT GROWTH PARAMETERS AND YIELDS

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7.1 Introduction

It has been known for a long time that soil salinity is directly correlated with the decline of agricultural crop productivity (Lauchli and Grattan, 2007). Even associated with all the scientific developments, these damages are still existing (Turkan and Demiral, 2008). Unpredictable climate pattern and leading anthropogenic soil pollution/disturbances significantly affects the soil fertility and paddy crop productivity. During last few decades many times farmers are experiencing huge loss of their crop yields due to uncertain climatic calamities. Thus, it is necessary to reduce anthropogenic mediated addition of agro-chemicals in agriculture fields to minimize the crop yield loss and to maintain the sustainable future crop cultivation.

Soil salinity has been found to negatively correlate with plant growth as unbalanced pH and soil conductivity adequately disturbs plant health and crop productivity. Application of FYM in agriculture to reduce the impact of soil salinity has been adopted by farmers from a long time. Furthermore, application of organic amendments in combination with efficient agriculturally important microbes like PGPR undoubtedly will improve the soil health and crop productivity (Vimal et al., 2018a). Therefore, the aim of present objective was also to find out correlation between soil physico-chemical properties, paddy plant growth parameters and antioxidant activities across different treatments (FYM and SRV4 strain) and years 2015 and 2016.

7.2 Material and Methods

7.2.1 Correlation Analysis between Soil Physico-chemical Properties, Paddy Plant Growth Parameters and Antioxidant Enzymatic Activities

The Pearson correlation analysis between, soil physico-chemical properties, plant growth parameters and antioxidant enzyme activities was performed using SPSS (Version 20: IBM, Armonk, NY, USA). This correlation analysis was performed on pool data (mean values) of physico-chemical properties, paddy plant growth parameters and antioxidant activities across different treatments and years. N=12 (4 treatments × 3 replicates).

7.3 Results

A negative correlation between soil physico-chemical properties and paddy plant growth parameters was observed on pool data (mean values) across different treatments and years (Table 7.1). The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant reduction in soil pH, EC, TSS and BD (Chapter 5 Table 5.1) of paddy soils, while organic-C available-N, -P, -K (Table 5.1) and paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields) (Chapter 6 Table 6.1) was enhanced significantly. This probably could be the reason for a negative relationship between soil physico-chemical properties and paddy plant growth parameters in this study (Table 7.1).

Table 7.1 Pearson's correlation (2-tailed) between soil physico-chemical properties and paddy plant growth parameters. This correlation analysis was performed on pool data (mean values) of physico-chemical properties and paddy plant growth parameters across different treatments and years. N=12 (4 treatments × 3 replicates).

Study parameters	**pH**	**EC**	**BD**	**TSS**	**Organic C**	**Available N**	**Available P**	**Available K**	**Plant height**	**Tiller number**	**Panicle number**	**1000 rice grain weight**
EC (ds m^{-1})	.751**											
BD (g cm^{-3})	.619**	.746**										
TSS (mg L^{-1})	.751**	1.000**	.746**									
Organic C (%)	-.642**	-.864**	-.593**	-.864**								
Available N (Kg ha^{-1})	-.728**	-.729**	-.502*	-.729**	.821**							
Available P (Kg ha^{-1})	-.800**	-.624**	-.495*	-.624**	.667**	.724**						
Available K (Kg ha^{-1})	-.573**	-.727**	-.400*	-.727**	.825**	.746**	.782**					
Plant height (cm)	-.671**	-.607**	-.351*	-.607**	.674**	.802**	.707**	.648**				
Tiller number ($Plant^{-1}$)	-.810**	-.650**	-.489*	-.650**	.776**	.784**	.902**	.736**	.803**			
Panicle number (m^2)	-.707**	-.673**	-.436*	-.673**	.792**	.793**	.860**	.852**	.821**	.869**		
1000 rice grain weight (g)	-.657**	-.663**	-.437*	-.663**	.822**	.811**	.799**	.785**	.780**	.865**	.895**	
Rice grain yields (t ha^{-1})	-.653**	.-693**	-.450*	-.693**	.855**	.855**	.789**	.804**	.805**	.852**	.892**	.962**

** Correlation is significant at the 0.01 level (2-tailed).
 * Correlation is significant at the 0.05 level (2-tailed).

The results of correlation analysis showed a negative relationship between soil paddy plant growth parameters and antioxidative enzymes activities when data were pooled across different treatments and years (Figures 7.1 and 7.2). The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant increase in paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields) (Chapter 6 Table 6.1, while antioxidant enzymatic activities (CAT, SOD, POX and APX) (Table 6.2) was reduced significantly. In this study, an increase paddy plant growth parameters and decrease in antioxidant enzymatic activities due to FYM and SRV4 treatments could be the reason for a negative relationship between soil paddy plant growth parameters and antioxidant levels (Figures 7.1 and 7.2).

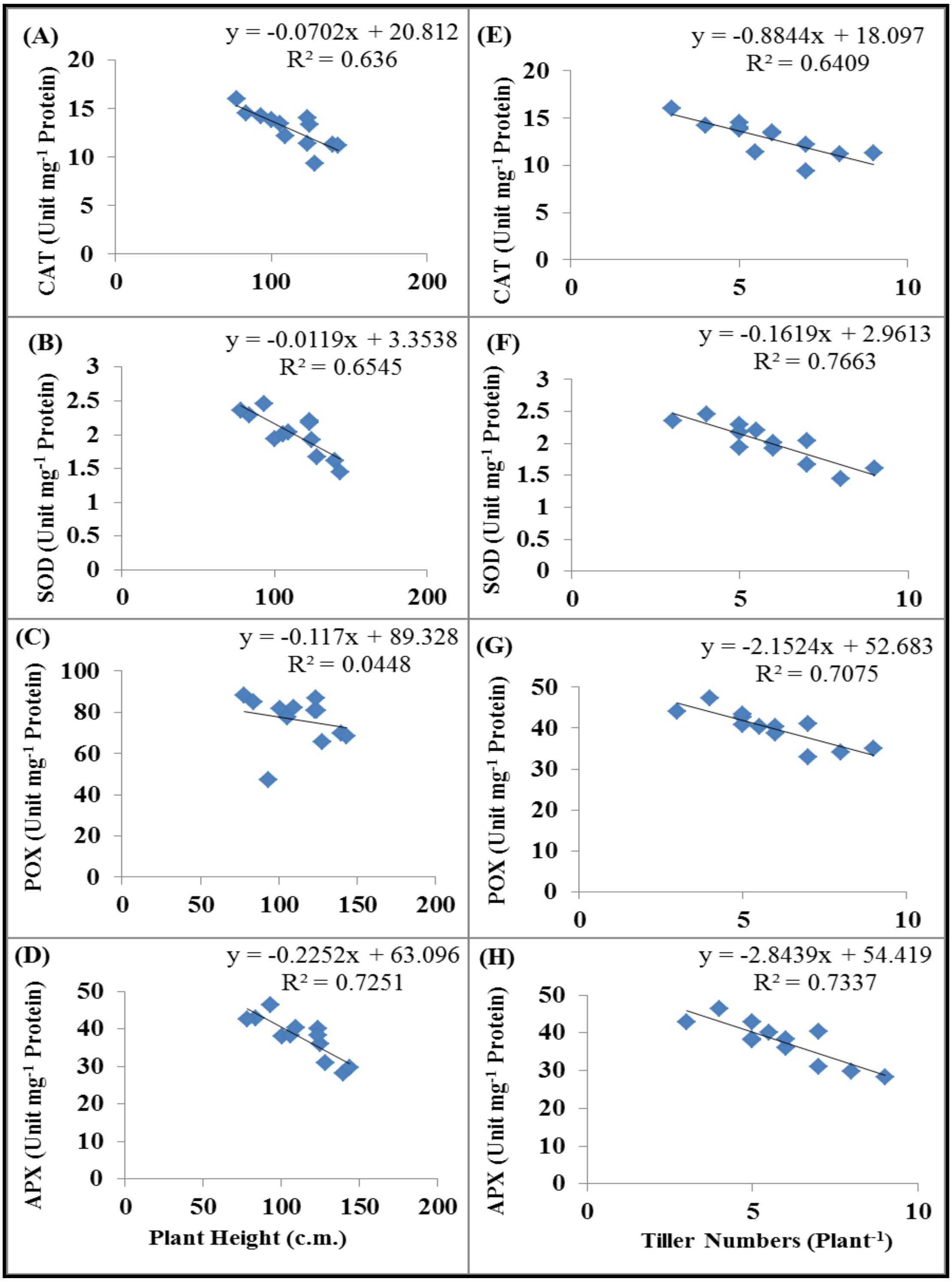

Figure 7.1 Regression analyses between plant height, tiller number and antioxidant activities. This correlation analysis was performed on pool data (mean values) of physico-chemical properties and paddy plant growth parameters across different treatments and years. N=12 (4 treatments × 3 replicates)

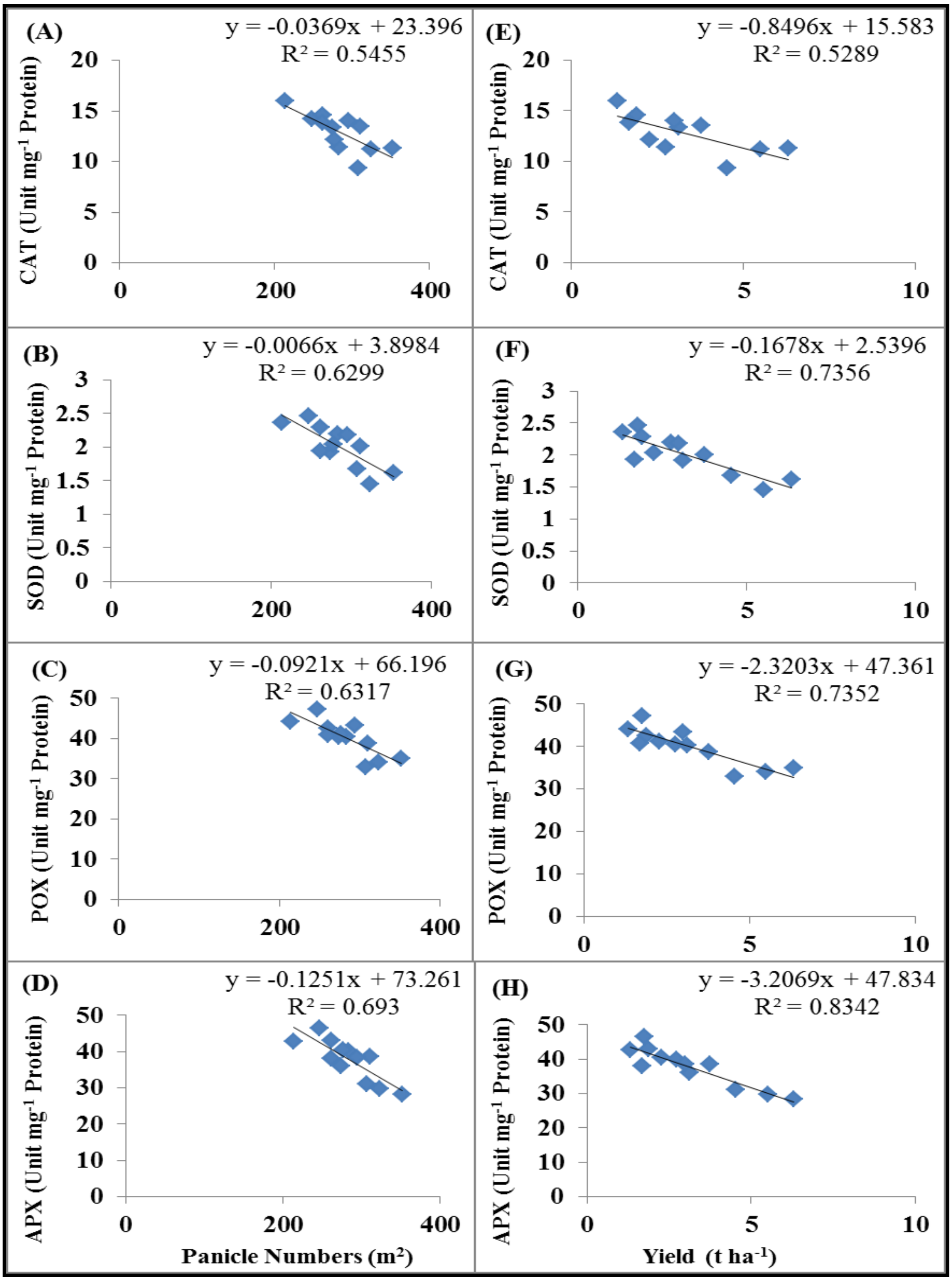

Figure 7.2 Regression analyses between panicle number, crop yields and antioxidant activities. This correlation analysis was performed on pool data (mean values) of physico-chemical properties and paddy plant growth parameters across different treatments and years. N=12 (4 treatments × 3 replicates)

7.4 Discussion

The results showed a linear negative relationship between soil physico-chemial properties, paddy crop growth parameters and antioxidant activities. As the data of present study indicated that the soil properties such as pH, EC, TSS and BD and antioxidative enzymatic activities (CAT, SOD, POX and APX) were significantly decreased due to FYM+SRV4 treatments in both years. But contrarily to the previous results, the organic-C, available nutrients and paddy plant productivity increased due to treatments in both study years. This indicates that paddy plants have developed defense enzymatic systems in untreated (control) soil and therefore, synthesized a greater amount antioxidative enzymes (CAT, SOD, POX and APX) to cope up the adverse situations and damage caused by salinity mediated oxidative stress (Upadhyay et al., 2012). Under salinity stress generation of excess ROS disturb plant health. Soils salinity leads to promote ROS production (Gill and Tuteja, 2010). Further, in the present experiment, a decline in antioxidant enzyme production to FYM+SRV4 treated soil in both years possibly is due to reduction in soil pH, EC and TSS due to acidic nature of FYM amendments. The decrease in EC is associated with decline in soil salinity. Decline in soil salinity level is positively correlated with reduced stress production. Similar trends of decline in antioxidant activities in plants under field experiments treated with FYM and organic amendments were observed (Singh et al., 2010 and 2013). It is suggested that application of potential salt tolerant microbes like *C. albidum* SRV4 strain, isolated from saline stressed soils to agro-ecosystem, will possibly be the best alternative to alleviate soil salinity and restoration of soil fertility of a disturbed paddy field (Singh et al., 2012; Vimal et al., 2018a).

7.5 Conclusions

Based on the above results and correlation analyses between soil physico-chemial properties, paddy crop growth parameters and antioxidant activities, it may be concluded that FYM and salt tolerant PGPR *C. albidum* SRV4 strain could be potential amendments to improve the soil properties and paddy crop productivity in saline soils. The FYM, due to presence of abundant amount of organic and inorganic nutrients and acidic properties, improves the soil condition and declines the soil salinity, while SRV4 strain as inoculant with plant growth promoting attributes enhances the paddy plant growth parameters and yields in saline soils. Positive relationship between paddy crop productivity components and soil parameters like organic-C and available-N are associated with reduction in salinity level in field conditions due FYM amendments. This field experiments possibly validate SRV4 strain potentiality in stress management and therefore, in association with FYM amendment it can play a vital role in restoration of soil fertility and considered as potential bio-tool to promote sustainable paddy agricultural management in salinity affected area of Lucknow and other similar regions.

CHAPTER 8

SUMMARY

CHAPTER 8

SUMMARY

Soil salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination density, plant vigour and crop yield, limiting nutrient absorption and reducing the quality of the available water. For example, elevated salinity weakens plants due to the increase in osmotic pressure and the toxic effect of the salts (Munns and Tester, 2008; Paul and Lade, 2014). Saline soils show the following physical-hydric characteristics: low permeability, low hydraulic conductivity and aggregate instability (Freire, 2009). Salinity also affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency (Netondo et al., 2004; Barnawal et al., 2017). Salt stress as one of the most widespread abiotic constraints in food production may also result in the negative ecological, social and or economic outcomes. Agricultural crops drastically affected in high salt concentration. High salt concentration lower down crop production and affect soil physiochemical and ecological balance of the ecosystem. Successful remediation of salt degraded areas for crop production, based on sustainable management practices evolving efficient, low cost, easily adaptable methods, is the challenge.

Rice (*Oryza sativa* L.) is a vital agent for nutritional security and according to report of International Rice Research Institute (IRRI)-Philippines, 90% of global production of rice is consumed by the Asian population. Food and Agriculture Organisation (FAO)-United Nations, data shows (*http://faostat3.fao.org*), after sugar cane the next three first crops in terms of production (million tonnes) in the world are the cereal maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum*

L.). In 2011, global production of rice was almost 723 MT. Seeds utilizes different prompts from the earth to terminate dormancy and where and when to set up another plant. Primary signals utilized by seeds for sprout as part of world compromise light, nutrients, suitable heats, water substance, and likely different signs. Undisputedly plants are flexible in environment with the possibility to build up a plenty of morphological patterns relying on growth conditions to which they are exposed. This morphological flexibility has empowered plants to colonize almost every edge of globe and to survive in the harshest conditions. Rice exhibits wide adaptability to different environments, which makes it the most widespread crop in the world. It can grow in saline condition, drought conditions or in shallow water (up to 50 cm of water), and in a wide range of latitudes and up to 3000 m altitude. For this reason, it is considered a strategic crop for food security in the world by the FAO. However methodologies have been utilized aiming at enhancing multiple stress resilience in early decades but inadmissible results occurs. Escalating salinity fields imposed severe osmotic damages on paddy plant and declined grain productivity. Applications of agrochemicals in rejuvenation of saline soils drastically deteriorate soil physicochemical and biological properties. Thus, it is now necessary to these Global concerns somewhat mitigated through upgraded sustainable options.

The emerging environmental issues such as soil pollution, land degradation, loss of soil fertility or soil microbial diversity, or rise of average global temperatures, among many others, are adversely impacting the various ecosystems (Singh and Gupta, 2018). The changes in plant and animal communities are relatively slow, but alterations in soil microbial community compositions and their ecological functioning happen much more rapidly, but mostly stay ignored. The soil microbial communities are the key responders to any environmental change, but details on what exactly happens to microbial community composition and their functional role as a response to variations in soil parameters changes to most of the cases are still quite unclear. Microbes, a tiny living with unmatchable capacity offer an innovative and feasible option in agriculture and got position of decent bioengineers for engineered tainted agro-ecosystems (Singh, 2015). Beneficial microbes associated with plants rhizosphere are known to stimulate plant growth and enhance plant resistance to biotic (diseases) and abiotic (salinity, drought, pollutions, etc.) stresses. Roots are the vital part of plant can taste which supplements are available in soil and answer is easily shown in plant health. They can also taste and integrate signalling by means of different chemicals substances that are delivered in their distinctive organs, as well as by microorganisms, plants, and animals in their environments (Cheynier et al., 2013; Fonseca et al., 2014). Root exudate varies with plant genotypes, recruit microbial partners towards and along roots (Patel et al., 2015). Rhizosphere microbial counts go beyond to 1×10^{11} microbial cells per gram root (Egamberdieva et al., 2008) in eutrophic while it has decline up to 10^4 under stressed terrestrial ecosystems. Microbes under rhizosphere can trustily modulate root exudate patterns (Patel et al., 2015), improves rhizospheric architecture (Vimal et al., 2017) and induce systemic resistance to consequent pathogen attack (Glick, 2014).

Plant-growth-promoting-rhizobacteria (PGPR), a key component of soil micro-biota, could play vital roles in the maintenance of plant fitness and soil health under stressed environments. The PGPR got special promotions among soil microbes during last few decades due to unmatchable capability. After successful tuning with the plant roots rhizobacteria utilizes carbohydrates, amino acids, organic acids and exercises with different plant growth promontory traits (Choudhury et al., 2014). PGPR boost plant vigor by means of different mechanisms, altering root architecture (Grobelak et al., 2015), initiate phytohormone levels (Glick, 2012), pathogen reduction (Singh et al., 2013), stress tolerance (Vimal et al., 2018a) and comprehension these unpredictable cross-kingdom interactions inspires us into root formative science and bacterial signaling (Singh et al., 2011; 2015; 2018a, b). The PGPRs are proficient in modulating the root system architecture which is a critical factor of productivity (Singh 2015). The capability of PGPR to influence plant development and root processes was excellently addressed by Hatesami and Maheshwari, 2018. By contrast, the mechanisms by which PGPRs up-regulate cell division, and improve the equilibrium between proliferation and differentiation in the primary root and lateral root initiation sites, remain largely unidentified (Veloccia et al., 2016). These adjustments are built up by changing plant endogenous signalling pathways. The PGPR got tremendous attention in to taking care of soil and plant health due to environmental calamities (Vimal et al., 2016; 2017; 2018a, b). The PGPR provides great promise in sustainable future soil fertility and crop productivity managements. The PGPR communications with their host plant have knocked the minds of researcher for advancement in PGPRs based microbial technology.

The application of organic manure as a soil conditioner in combination with suitable salt tolerant PGPR strains could improve the soil-plant-microbe interaction and may enhance the crop yield under stressed soil conditions. Countless number of microbes as algae, mycorrhizal fungi, PGPR processes in different nutrient cycling. Thus amendments of organic manures in stressed soils as bio-fertilizer, compost, and vermi-composts offers a blameless bio-agent in rejuvenation of degraded ecosystem.The PGPR in association with organic amendments like FYM may significantly reduces the amount of energy demanding inputs, such as chemical fertilizers and can contribute to mitigation and adaptation for paddy plant under saline soils. Investigations are needed in this area that how and how much of the PGPR with

FYM amendments facilitates plant growth, enhanced paddy crop production and regenerate soil fertility. Thus, the present doctoral research hypothesizes that the addition of salt tolerant PGPR inoculant + FYM (as organic amendment) to paddy soil could be a novel and cost-effective tool to enhance soil fertility and alleviate the salinity stresses of paddy crop plants. The present study was conducted to investigate the efficacy of salt tolerant PGPR strain + FYM on paddy plant growth productivity with following 3 objectives.

(i) To analyze the impact of PGPR and farmyard manure (FYM) applications on soil physico-chemical properties.

(ii) To examine the impact of PGPR and FYM applications on plant growth parameters and antioxidant level in paddy crop in saline soil.

(iii) To assess the correlation between the PGPR and FYM application and plant growth parameters and yields.

Note: For more clarity it is important to mention here that whole experimental work of present study broadly may be divided into two parts.

Part I	**Part II (3 objectives)**
Isolation and characterization of salt tolerant plant growth promoting rhizobacteria from saline soils (Chapter 3)	Application of isolated salt tolerant most efficient PGPR strain *C. albidum* SRV4 in combination with FYM in field conditions (Chapters 4, 5, 6 and 7)

Part I

Twenty nine promising halo-tolerant PGPRs were isolated and screened for their PGP traits from the rhizosphere of naturally growing plants of saline soils located at Sandila region of Hardoi, Uttar Pradesh, India. Out of 29 isolates, one most efficient salt-tolerant isolate having potential PGP attributes, was selected for further study. Based on 16S rRNA gene sequencing and BLASTn analysis, the isolate was identified as *Curtobacterium albidum* SRV4 strain. The SRV4 expressed positive attribute for nitrogen (N_2) fixation, exopolysaccharide production (EPS), hydrogen cyanide (HCN), Indole-3-acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. The higher doses of NaCl negatively affected paddy plant physiology and growth parameters. Paddy plants in pot experiment treated with SRV4 showed significant differences ($P < 0.001$) in improvement in plant growth parameters, photosynthetic pigment efficiency, membrane stabilization index and proline content. A significant variation ($P < 0.001$) in enhancement in antioxidative enzymatic activities catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) and K^+ uptake in paddy plants was noted due to *C. albidum* SRV4 inoculation. The *C. albidum* SRV4 has been found as effective microbial agent to improve photosynthetic efficiency, modulation of osmolytes and antioxidative enzymes due to efficient PGP attributes, development of induced systemic tolerance and alleviating salt stress in paddy plants. The sequence data of *C. albidum* SRV4 has been deposited to the GenBank nucleotide sequence database with the accession number KX 81071.

The above isolated and identified salt tolerant PGPR *C. albidum* SRV4 strain was used as microbial inoculant in combination with FYM for further paddy crop field experiment.

Part II

Experimental Field Design and Paddy Crop Cultivation

The field experiment was conducted for two successive paddy crop cycles in the year 2015 and 2016, at field experimental station, Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India. For both the study years, 2015 and 2016, four treatments used in this experiment were: (a) Control, (b) *C. albidum* SRV4 strain, (c) FYM (50 t ha^{-1}) and (d) FYM (50 t ha^{-1}) + *C. albidum* SRV4 strain. Therefore, total 12 experimental plots (each having 3m×2m dimensions) were established in completely randomized block design (CRBD) with each treatment having three replications.

For present investigation, the selected experimental crop was paddy (*Oryza sativa*). The rice variety namely HUR 3-4 (Hindu-University-Rice 3-4) was obtained from Department of Genetics and Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University (South campus), Mirzapur, Uttar Pradesh. The rice seeds sterilization, inoculum preparation and application to paddy field were done as described in Chapter (3). The nursery of rice cultivar was prepared in month of June during both the years 2015 and 2016.

Five paddy hills having equal height was transplanted in month for both the years 2015 and 2016 at 20cm×20cm dimensions in each plot. A total of 600 paddy hills were transplanted in each plot. The SRV4 inocula were sprayed on paddy plant parts on panicle initiation stage at the rate of 2 litres $plot^{-1}$. The matured paddy crop was harvested during the month of November in both years 2015 2016.

Objective 1: To Analyze the Impact of PGPR and Farmyard Manure (FYM) Applications on Soil Physico-Chemical Properties

The results of different FYM+SRV4 amendments on soils physico-chemical showed significant ($P < 0.001$) variations due to treatments. The application of FYM significantly reduces the pH and EC, BD and TSS level of paddy soils in both the study years 2015 and 2016. However, improvement in Organic-C, available-N, available-P and available-K was observed in FYM+SRV4 treated plots during both the crop cultivation years. Based on the results, this study suggests that application of FYM in combination with PGPR *C. albidum* SRV4 had significant impact on soil conditions due to beneficial activities of plant growth promoting inoculant and under salinity stress.

Objective 2: To Examine the Impact of PGPR and FYM Applications on Plant Growth Parameters and Antioxidant Level in Paddy Crop in Saline Soil

Paddy plant growth variables (plant height, tiller number, panicle number) and rice grain yields was noted maximum in SRV4+FYM amended plots compared to other treatments. ANOVA showed significant variations in paddy plant growth variables ($P < 0.001$) and rice grain yields ($P= < 0.01$ to < 0.001) due to treatments in both the years 2015 and 2016. Two-way ANOVA for the pooled data of paddy plant growth variables and rice grain yield across different treatments and years showed that impact of treatment was only significant ($P < 0.001$), while impact of years and treatment × year interaction was not significant. Across different treatments maximum paddy plant height in both the years 2015 (135±6.77 cm) and 2016 (139±7.68 cm) was noted in FYM+SRV4 amended plots compared to other treatments. The maximum tiller numbers per paddy plant were appears in FYM+SRV4 amended plots in both crop cultivation years. Similarly the panicle numbers were recorded maximum in FYM+SRV4 amended plots in 2015 and 2016. For both the years, also the weight of 1000 rice grain was found maximum in FYM+SRV4 amended plots compared to other treatments. The maximum rice grain production was found in FYM+SRV4 amended plots for 2015 (5.04±0.53 t ha^{-1}) and 2016 (5.85±0.57 t ha^{-1}). It is suggested that the efficient salt tolerant PGPR SRV4 strain isolated from saline soils with FYM amendments (50 t ha^{-1}) nourishes paddy degraded soils, enhances availability of nutrients, modulates plant antioxidants and improves plant health and ultimately the paddy crop yield.

Antioxidant enzyme activities (CAT, SOD, POX and APX) were found lowest in SRV4+FYM amended plots and highest in control plots. ANOVA showed significant variations ($P= < 0.01$ to < 0.001) in antioxidant enzyme activities in both the years 2015 and 2016 during paddy crop cycles. When the data of antioxidant enzyme activities were polled across different treatments and years, two-way ANOVA showed significant differences ($P < 0.001$) only for treatments, while impact of years and treatment × year interaction was not significant. In the present experiments, an enhanced antioxidative enzymes production in paddy plants of control plots, possibly could be to neutralize the adverse impact of higher salinity stress and is in conformity with the results of other investigations.

Objective 3: To Assess the Correlation between the PGPR and FYM Application and Plant Growth Parameters and Yields

To find out the relationship between, soil physico-chemial properties, paddy crop growth parameters (plant height, tiller number, panicle number and rice grain yields) and antioxidant enzyme activities (CAT, SOD, POX and APX), across different treatments and years, pooled data were subjected to regression analysis. A negative correlation between soil physico-chemical properties and paddy plant growth parameters was observed across different treatments and years. The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant reduction in soil pH, EC, TSS and BD of paddy soils, while organic-C available-N, -P, -K (Table 5.1) and paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields) was enhanced significantly. This probably could be the reason for a negative relationship between soil physico-chemical properties and paddy plant growth parameters in this study.

The results of correlation analysis showed a negative relationship between soil paddy plant growth parameters and antioxidative enzymes activities when data were pooled across different treatments and years. The results of present study showed that input of FYM and SRV4 strain, alone or in combination, resulted in significant increase in paddy plant growth parameters (plant height, tiller and panicle numbers and rice grain yields), while antioxidant enzymatic activities (CAT, SOD, POX and APX) was reduced significantly. In this study, an increase paddy plant growth parameters and decrease in antioxidant enzymatic activities due to FYM and SRV4 treatments could be the reason for a negative relationship between soil paddy plant growth parameters and antioxidant levels.

Conclusions

The aim of the present investigation was to isolate and identify the efficient PGPR strains from the saline soils that could be evaluated for improve the growth and yield of paddy crop under salinity stress. The seeds inoculated with SRV4 strain increased paddy plant growth, yields compared to the non-inoculated controls. The paddy yield components were enhanced even more, when rhizobacteria treated seedlings was transplanted in FYM amended plots. Significant variation confines fertility rejuvenation, crop production and plant antioxidant capacity. In general a negative correlation between crop growth parameters and antioxidant enzyme activities were observed. The negative correlations between plant growth parameters and antioxidant enzymes levels in the present investigation indicated that inoculation of salt tolerant C. albidum SRV4 to soil reduces the salinity stress in paddy plants. These results suggest that higher antioxidative enzyme requirement by paddy plant at higher salinity levels, could play as important role to protect plants against oxidative injury. Based on the above results, this study suggests that inoculation of C. albidum SRV4 had significant impact on paddy growth due to their plant growth promoting attribute under salinity stress. With reference to the combined effect of microbial inoculants and their survival under salinity stress and their competition with other indigenous microbial flora, it would be more effective and viable to apply the locally well adapted salt tolerant native microbial agents like C. albidum SRV4 to ensure the best results on paddy crop productivity under salinity stress. This field experiments possibly validate SRV4 strain potentiality in stress management and therefore, in association with FYM amendment it can play a vital role in restoration of soil fertility and considered as potential bio-tool to promote sustainable paddy agricultural management in salinity affected area of Lucknow and other similar regions.

Although, the ecologically sustainable paddy agricultural practices are essential to contribute significantly for food security, and efficient agriculturally important microbes (PGPR inoculants) are playing very crucial role in high paddy and others crop production due to their immense plant growth promoting attributes. Nowadays, bio-formulations (microbes or their metabolites) use in combination with organic amendments has been considered as promising means for crop sustainability in stress soil conditions. Inoculation of agriculturally beneficial microbes along with their metabolites in agriculture fields, may be a potential option, proving more eco-friendly with multidimensional roles and consequently, demonstrating the new opportunity to develop the microbial-formulations for safe and sustainable agriculture. These sustainable crop productions are possible because of their better adaptability and survival under stresses, and ultimately that result in attenuating the chemicalization (pesticides/fertilizers use) in agriculture sector. However, the eco-friendly viable microbial-formulations progress at commercial level and constraints associated with the techniques are not yet explored in detail. It should be always kept in mind that the unpredictable biotic and abiotic edaphic factors that determine the survival and potential of the microbial inoculants responses and functioning after their delivery in the field conditions. As most plant-inoculants interactions after addition in the field conditions are as yet unexplored and high-throughput sophisticated molecular tools/techniques are limited, so we only can predict that plant-microbes inoculants could be a general strategy in benefits of soil fertility. Therefore, there is need to assess critically such non-target special effects of microbial inoculants at broad level, and to validate such consequences before their delivery in the natural field conditions.

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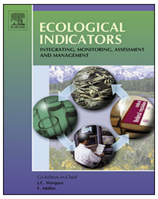

Original Articles

Plant growth promoting *Curtobacterium albidum* strain SRV4: An agriculturally important microbe to alleviate salinity stress in paddy plants

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ABSTRACT

Present study examined the inoculation of halo-tolerant plant-growth-promoting-rhizobacteria (PGPR) on paddy plant growth parameters and physiology under different salinity (0, 100, 200 and 300 mM) conditions. Twenty-nine promising halo-tolerant PGPRs were isolated and screened for their PGP traits from the rhizosphere of naturally growing plants of saline soils located at Sandila region of Hardoi, Uttar Pradesh, India. Out of 29 isolates, one most efficient salt-tolerant isolate having potential PGP attributes, was selected for further study. Based on 16S rRNA gene sequencing and BLASTn analysis, the isolate was identified as *Curtobacterium albidum* SRV4 strain. The SRV4 expressed positive attribute for nitrogen (N_2) fixation, exopolysaccharide production (EPS), hydrogen cyanide (HCN), Indole-3-acetic acid (IAA), and 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. The higher doses of NaCl negatively affected paddy plant physiology and growth parameters. Paddy plants treated with SRV4 showed significant differences ($P < 0.001$) in improvement in plant growth parameters, photosynthetic pigment efficiency, membrane stabilization index and proline content. A significant variation ($P < 0.001$) in enhancement in antioxidative enzymatic activities catalase (CAT), superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) and K^+ uptake in paddy plants was noted due to *C. albidum* SRV4 inoculation. Paddy plant growth parameters (plant root and shoot length, dry weight and tiller number) and antioxidative enzymatic activities were negatively correlated. In conclusion, *C. albidum* SRV4 has been found as effective microbial agent to improve photosynthetic efficiency, modulation of osmolytes and antioxidative enzymes due to efficient PGP attributes, development of induced systemic tolerance and alleviating salt stress in paddy plants.

1. Introduction

In agriculture sector, rice (*Oryza sativa* L.) has been considered as the vital staple food for billions of people, and provides nutritional food to one-half of the world's population (Nakbanponte et al., 2014). But paddy crop has been categorised as a 'thirsty' crop type as it requires enough water during the crop cycle (Thakur et al., 2016). Consequences of rise in human population, about 536–551 million tons of global rice demand is expected after a decade. Among different cereal crops, paddy rice, one of the highly consumed cereals, is chiefly cultivated by developing countries. However, during paddy cultivation in plane or flooded regions, the water added to the paddy soil is used by the crop plants, percolates downward or evaporates directly to the atmosphere. After evaporation, huge amount of salt accumulates on the soil surface. In flooded paddy fields, development of soil salinity due to accumulation of sufficient amount of salts on the soil surface could adversely affect plant nutrient uptake; diminish microbial growth, crop yields and soil fertility. Thus the paddy crop may be considered as the excellent model crop for the study of its physiological/biochemical parameters under salinity stresses (Khan and Hemlata, 2016).

Soil salinization of productive agriculture lands, principally with salt (NaCl), is most persistent and detrimental to paddy crop productivity. High salt concentrations accumulate both Na^+ and Cl^- simultaneously, although the effects of these two ions may vary (Nakbanponte et al., 2014). Na^+ accumulation declines soil porosity, soil aeration and water conductance (Porcel et al., 2012). High Na^+ ions also interfere with K^+ and Ca^{2+}, affect stomatal regulation, photosynthesis and enzymatic activities (Paul and Lade, 2014). Salinity diminishes a number of paddy plant growth parameters including panicles initiation, tiller number, spikelet formation, delayed heading and grain size (Nakbanponte et al., 2014). Deterioration of soil physico-chemical conditions due to salinity thus hinders crop plant nutrient uptake from soils, and ultimately, its productivity (Paul and Lade, 2014).

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Plant-microbe interactions play vital roles in the maintenance of plant and soil productivity under stress environment (Singh, 2015; Vimal et al., 2017). Stressed soil conditions adversely influence the microbial community composition and their population size in agriculture soils (Vimal et al., 2017). Under such soil conditions, plants construct favourable rhizospheric conditions by recruiting and attracting the beneficial microbial communities by secretion of root exudates- sugars, amino acids, organic acids and signalling molecules (Singh, 2015; Tiwari and Singh, 2017)). The favourable rhizospheric conditions may harbour enough beneficial microbial agents and in turn, the microbes provides several plant growth promoting benefits to crop plants (Egamberdieva et al., 2008; Mendes et al., 2011). Plant growth promoting rhizobacteria (PGPR) in close association with the root zones of saline soils, invite strong attention and attracts researchers owing to their versatility, survival and unmatchable plant beneficiary attributes under harsh soil conditions (Cardinale et al., 2015; Vimal et al., 2017). The PGPR promote plant growth through various mechanisms such as bio-film formation (Petrova and Sauer, 2016), phytohormones production (Barnawal et al., 2014), nutrients management (Singh et al., 2011) and antioxidants production (Upadhyay et al., 2012). However, the unknown mechanisms performed by PGPR under different stress environmental conditions such as soil salinity (Barnawal et al., 2014), drought (Mayak et al., 2004; Sandhya et al., 2009), metal contamination (Grobelak et al., 2015) and biotic stresses (Barnawal et al., 2017), are also reported in favour of crop health. Inoculation of PGPR directly to soils or bio-priming of seeds and seedlings with the PGPR, has been found to enhance seed germination and viability of seedlings in salt affected soils (Mayak et al., 2004; Nautiyal et al., 2013).

To survive various abiotic and biotic stresses, plants have evolved well developed antioxidative systems (Gill and Tuteja, 2010). Potential PGPR strains are also reported in the management of stresses by harmful radicals produced by plants during metabolism (Upadhyay et al., 2012). Antioxidants and antioxidative enzymes in plants, inoculated with PGP microbial agents, have been reported to scavenge the harmful radicals and minimize the stress induced damages (Khan et al., 2016). Antioxidants not only act as the compatible osmoprotectants but also serve as signalling molecules to modulate the osmotic balances of cell cytoplasm, and trigger the expression of specific genes, essential for the wellbeing of plant physiology and metabolism (Cui et al., 2016). It appears that PGPR could be an important group of beneficial microbes that can be exploited to alleviate the abiotic and biotic stresses in crop plants. Therefore, addition of salt tolerant microbial inoculants to the soil, could be the novel and cost-effective alternative to alleviate salinity stress in paddy crops. The isolation and identification of new salt tolerant PGPRs strains can be exploited to develop effective and viable bio-inoculants to enhance paddy production in salinity affected areas. There are scanty reports on application of PGPR inoculants to alleviate salinity stress, particularly the role of *Curtobacterium* sp. on paddy crop. Hence, the present study was conducted to investigate the efficacy of salt tolerant PGPR strains isolated from saline soils on paddy plant growth promotion under salt stresses. The most efficient isolate *C. albidum* SRV4 was selected on the basis of its *in vitro* PGP activity and 16S rRNA gene sequencing for application. A greenhouse experiment was conducted to examine the effect of SRV4 inoculation on plant growth parameters and antioxidant activities of paddy plants under salinity.

2. Material and methods

2.1. Isolation of salt tolerant rhizobacteria

The rhizospheric soil samples were collected from the salinity affected soils of Sandila, Lucknow district (latitude 20°07′ N, longitude 80°52′ E), India. The electrical conductivity (EC) and pH of the collected soils were 6.8 $dS\,m^{-1}$ and 8.1, respectively. Soil samples were serially diluted up to 10^{-6} and plated on nutrient agar (NA) (up to 1800 mM NaCl salt) and incubated for 48–72 h (28 ± 2 °C). The pure rhizobacterial cultures were stored at 4 °C for determination of PGPR activity, inoculum formulation and seed treatment.

2.2. Plant growth promoting attributes of isolated salt tolerant rhizobacteria

Nitrogen fixation efficiency was determined with Jensen's N free medium (Jensen, 1954). The isolated clones were streaked on Jensen agar g/L: $C_{12}H_{22}O_{11}$ (20.0), $K_2HPO_{4,}$ (1.0), $MgSO_4.7H_2O$ (0.5), $FeSO_4.H_2O$ (0.1), NaCl (0.5), Na_2MoO_4 (0.005), $CaCO_3$ (2.0), agar (15) and incubated for 48 h (28 ± 2 °C). Bacterial growth was observed as the qualitative evidence of N_2-fixation.

Phosphate solubilization activity was determined according to Pikovskaya (1948). The isolates were point inoculated on Pikovskaya's agar medium g/L: $(Ca_3)_2PO_4$ (5.00), $(NH_4)_2SO_4$ (0.5), NaCl (0.2), KCl (0.2), $MgSO_4.7H_2O$ (0.1), $MnSO_4.2H_2O$ (0.002), $FeSO_4.7H_2O$ (0.001), $C_6H_{12}O_6$ (10.00), yeast extract (0.5), agar (15.00) and incubated at 28 ± 2 °C for 5–7 days. The plates were observed for the clearance zone around the bacterial colony that indicated P solubilization. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone (Gaur, 1990).

Siderophore production efficiency of strains was carried on Chrom-Azurol S (CAS) medium containing [**Solution 1:** 60.5 mg CAS dye + 1 mM $FeCl_3.6H_2O$ prepared in 10 mM HCl, mixed with 50 mL doubled distilled water (DDW). Then, dissolved 72.09 mg Hexadecyltrimethylammonium bromide (HDTMA) in 40 mL DW and mixed with CAS solution. **Solution 2:** 30.2 g piperazine-N,N′-bis (2-ethanesulfonic acid (PIPES) dissolved in 800 mL DDW and maintained the pH at 6.8, then added, with 15.00 agar g/L. **Solution 3:** 70 mL solution containing glucose (2.00 g), mannitol (2.00 g), $MgSO_4.7H_2O$ (493.00 mg), $CaCl_2$ (11.00 mg), $MnSO_4.H_2O$ (1.17 mg), H_3PO_4 (1.4 mg), $CuSO_4.5H_2O$ (0.04 mg), $ZnSO_4.7H_2O$ (1.2 mg) and $Na_2MoO_4.2H_2O$ (1.00 mg). **Solution 4:** 30.00 mL of 10% casamino acid (Millipore filter sterilized)]. A drop of raised broth culture was spotted on CAS plates and incubated at 28 ± 2 °C for 72 h. Formation of orange halos around bacterial colonies showed that bacterial isolate produced siderophore (Schwyn and Neilands, 1987).

Hydrogen cyanide (HCN) production was determined by the method of Lorck (1948) with slight modifications. Freshly grown cultures were streaked on Kings'B agar medium containing g/L: Protease peptone (20.00), K_2HPO_4 (1.5), $MgSo_{4.}7H_2O$ (1.5), agar (20.00) and supplemented with 15.00 mL of glycerol. A filter paper saturated with 1% solution of picric acid and 2% Na_2CO_3 was placed on the lid of Petri dish. The Petri dish was sealed with paraffin tape and incubated at 28 ± 2 °C (4 days). Change in colour of filter paper from yellow to reddish brown was observed for cyanogenic activity.

Exopolysaccharide (EPS) production was assayed qualitatively with method of Nicolaus et al. (1999). Bacterial strains were grown in a minimal media containing g/L: $Na_3C_6H_5O_7$ (3), KCl (2), $MgSO_4.7H_2O$ (20), $MnCl_2.4H_2O$ (0.036), $FeSO_4.7H_2O$ (0.05), casamino acids (7.5), yeast extract (10) pH (7.2–7.8) for 5 days at 150 rpm at 28 ± 2 °C in 250 mL Erlenmeyer flasks each containing 100 mL broth supplemented with 5% NaCl. Supernatants were collected by centrifugation at ($10{,}000 \times g$) for 10 min at 4 °C. Cold absolute ethanol (3-fold) was then added dropwise under stirring, and the formation of a precipitate was considered positive for the production of exopolysaccharides (Siddikee et al., 2011).

Indole-3-acetic acid (IAA) production was determined with slight modification in method of Gordon and Weber (1951). Pure bacterial strains were inoculated in Nutrient Broth medium containing g/L: peptone (5.00), beef extract (3.00), NaCl (5) and incubated on orbital rotary shaker (Scigenics-Biotech, India) 72 h 120 rpm at (28 ± 2 °C). After 3 days, 2 mL of each culture was pelleted by centrifugation ($6000 \times g$) (Remi CPR-30 Plus) and the supernatant was discarded. Cell pellets were washed with 1 mL of PBS and re-suspended in phosphate buffer saline (PBS). About 1 mL of cell suspension (corresponding to cell density of 10^7 cells/mL) was added to 10 mL of nutrient broth amended with tryptophan (100 μg/mL) and incubated at 28 ± 2 °C with continuous shaking at (120 rpm) for 48 h. After incubation, 2 mL of bacterial culture was centrifuged at (12,000 × *g*) for 10 min. After this, about 1 mL of supernatant was transferred to a fresh tube in which 100 μg/mL of 10 mM orthophosphoric acid and 2 mL of Salkowski's reagent (1 mL of 0.5 M $FeCl_3$ in 50 mL of 35% $HClO_4$) were added, and incubated for 30 min in dark at room temperature. Development of pink colour indicated IAA production. Meanwhile, IAA concentration was quantified calorimetrically method at 530 nm using spectrophotometer (Evolution 201 UV–Vis Spectrophotometer) and calculated by comparing with the standard curve prepared with crude IAA.

The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity of isolates was assayed according to Penrose and Glick (2003). All 29 isolates were screened for their ability to utilize 1-aminocyclopropane-1-carboxylate (ACC) (3 mM) as the sole nitrogen source in Dworkin Foster (DF) minimal medium (Dworkin and Foster, 1958) g/L containing KH_2PO_4 (4), Na_2HPO_4 (6), $MgSO_4.7H_2O$ (0.2), glucose (2.00), gluconic acid (2.00), citric acid (2.00), agar (15.00) and trace elements mg/L [$FeSO_4.7H_2O$ (1.00), H_3BO_3 (10), $MnSO_4.H_2O$ (11.19), $ZnSO_4.7H_2O$ (124.6), $CuSO_4.5H_2O$ (78.22), MoO_3 (10.00), FeSO4.7H_2O (1000)] at pH 7.2 according to method of Dell'Amico et al. (2005) with some modifications. The culture conditions applied on DF salt minimal medium (Dworkin and Foster, 1958) alone was considered as the negative control and DF salt minimal medium with $(NH_4)_2SO_4$ (2.0 g L^{-1}), the positive control. The quantification of ACC deaminase activity was done by measuring the amount of α-ketobutyrate generated as the product by of the reaction spectrophotometrically (UV–Vis Spectrophotometer SPECORD-50 Plus) at 540 nm (Barnawal et al., 2017). The activity of ACC deaminase by rhizobacterial strain is expressed in nmol α-KB mg^{-1} protein h^{-1}.

2.3. Molecular characterization of rhizobacterial isolate

The potent salt tolerant rhizobacteria strain was identified by 16s rRNA gene (rDNA) sequence analysis. Total genomic DNA was isolated from the log phase of bacterial culture. The quality and quantity of the DNA was analysed using agarose gel electrophoresis and Nanodrop ND-1000 Spectrophotometer. The universal primer 27F (5′AGAGTTTGAT CCTGGCTCAG3′) and 1492R (3′ACGGCTACCTTGTTACGACTT5′) was used to partially amplify the 16s rRNA encoding gene from the rhizobacteria (Edwards et al., 1989). Amplification was performed in 25 μL final volume containing 2 μL genomic DNA (100 ng), 1.5 μL each of forward and reverse primer, 2.5 μL 10× Taq polymerase buffer, 2.5 μL dNTPs, 0.4 μL Taq, 2 μL $MgCl_2$ (250 mm), 1.25 μL $(CH_3)_2SO$ (5%), 0.80 μL BSA and 10.55 μL of Milli-Q water (MQ) (Weisburg et al., 1991). PCR conditions consisted of initial denaturation step at 95 °C (5 min), 31 amplification cycles of denaturation at 94 °C (1 min), annealing at 57.4 °C (1 min) and primer extension at 72 °C (2.30 min); followed by a final extension at 72 °C (15 min) with thermocycler (modified from Awasthi et al., 2011). Aliquots of the PCR products were analysed in 1.5% (w/v) agarose gels by horizontal gel electrophoresis. The amplified product was purified with the PCR Clean-up Kit (Axygen) according to manufacturer's protocol, and sequenced via universal primers and Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) on a 3130 × l Genetic Analyzer (Applied Biosystems, USA).

2.4. Sequence analysis

Sequence analysis was completed using the nucleotide BLAST (BLASTN) (http://www.ncbi.nlm.nih.gov/BLAST) on the National Centre of Biotechnology Information (NCBI) website. Phylogenetic and molecular relatedness of the 16S rRNA sequences of *C. albidum* SRV4 and its related species were shown using software MeGAlign 6.0 and align using CLUSTALW. The nucleotide sequence data have been deposited with NCBI.

2.5. Application of isolated salt tolerant C. albidum SRV4 on paddy plant

2.5.1. Inoculum preparation and paddy seed treatment

To produce the bacterial strain inocula, single colony of isolates was inoculated in 100 mL nutrient broth and incubated on orbital rotary shaker (Scigenics-Biotech, India) at 150 rpm for 48 h (28 ± 2 °C). The culture obtained at exponential phase was centrifuged (6000 × *g*) (Remi CPR-30 Plus) for 10 min (4 °C) and bacterial cells washed and re-suspended in 100 mL of 0.85% (NaCl) saline solution. The bacterial cell density was maintained up to 1×10^8 CFU mL^{-1}. The enumeration and calculations of bacterial cell density were carried out following the drop method (Hoben and Somasegran, 1982). Briefly, 1 mL of pellet suspension was used to prepare 10-fold serial dilutions (10^{-2}–10^{-9}). In the final volume of 1 mL, about 10 μL of each dilution were placed on nutrient agar plates. After 24 h of incubation, growth was recorded and cell density determined considering data from the last dilution in which bacterial growth was achieved.

For present study, the paddy cultivar (*Oryza sativa* L.) HUR 3-4 was procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, South Campus (Barkachha), Mirzapur, India. Seeds were washed with demineralized water and treated with 2% sodium hypochlorite solution for 10 min for surface sterilization. The sterilized seeds were soaked in double volume of bacterial suspension (1×10^8 CFU mL^{-1}) and kept at 28 ± 2 °C on a rotary shaker (90 rpm) for 9 h to facilitate the penetration of the bacterial inoculum inside the seeds. Carboxy methyl cellulose (CMC) (2%) was added to the suspension as a sticker. After 9 h of inoculation, paddy seeds were dried in shade for use in further experiments. Solitary sterilized seeds were used as control.

2.5.2. Pot experiment and paddy plant growth parameters

The experiments were conducted in earthen pots (triplicate) independently under greenhouse during July–September 2015. The treated as well as un-treated (control) paddy seeds were allowed to germinate in sterilized Petri dishes at 28 ± 2 °C in BOD incubator (Remi CI-12 Plus) for 12 h day/night cycle (8 Watt fluorescent light) for 4 days. The germinated seedlings were transplanted in earthen pots containing sterilized soil (pH 7.1, EC 3.2 dS m^{-1}, Organic-C 0.14%, organic matter 0.24%, available N 98 kg h^{-1}) and cultivated for 25 days in greenhouse. After 25 days of cultivation, before transplantation, the root dipping mechanism was adopted to introduce the bacterial inoculum to the paddy seedlings according to Nandkumar et al. (2003). Five paddy seedlings per hill having equal height, and three hills per pot were transplanted in earthen pots containing 9 kg of non-sterile field soil and farm-yard-manure (FYM) in 3:1 ratio. A blanket application of NPK fertilizer (150:80:40 kg^{-1} soil) was also applied as the basal dose. The earthen pots with paddy plants treated with salt (NaCl) and without bacterial inoculation served as positive control, while pots without NaCl and bacterial culture were considered as negative control. To asses that impact of different doses of NaCl (0, 100, 200 and 300 mM) on paddy plants planted in earthen pots 750 mL of NaCl solutions were applied for each treatment. To achieve the final NaCl dose (100, 200 and 300 mM) for the respective treatment (except control) in earthen pots, the concentration of NaCl solution was increased gradually. After seven weeks, the paddy plants exposed to various salinity regimes were harvested on September 15, 2015 for growth parameter measurements and antioxidant enzymes analyses.

During paddy cultivation in earthen pots, to test the viability and survival of inoculated SRV4 strain, total CFU counts of rhizospheric soil of inoculated paddy plant were also monitored at the time of plant harvesting. Maximum bacterial colonization (7.0×10^7 CFU) was recorded in 0 mM NaCl and minimum (4.3×10^7 CFU) at 300 mM NaCl dose. After seven weeks of different treatments, paddy plants were harvested to determine the plant height, shoot length, root length and number of tillers per hill. Plant dry weight was determined through oven dried plant samples at 65 °C (72 h).

2.6. Effect of bacterial isolate on paddy plant performance under salinity

Chlorophyll a (*Chl a*), chlorophyll b (*Chl b*), total chlorophyll (*Chl a + b*) and carotenoids were examined spectrophotometrically with modified methods of Wellburn (1994). Fully expanded leaf 0.5 (g) samples were dipped overnight (12 h) in 85% acetone for the extraction of chlorophyll pigments. The supernatant taken was centrifuged (6000 × *g*, 5 min) at 4 °C (Remi CPR-30 Plus) and diluted with the same concentration of acetone for spectrometric measurements. The pigment contents were calculated at absorbance at 452.5, 644, 663 nm alongside blank of untainted acetone (85%). *Chl a, b* and total chlorophyll and carotenoids were estimated using formula:

$$Chl\ a(\mathrm{mg\ g^{-1}\ FW}) = 10.3 \times \ A_{663}{-}0.98 \times \ A_{644} \quad (1)$$

$$Chl\ b(\mathrm{mg\ g^{-1}\ FW}) = 19.7 \times \ A_{644}{-}3.87 \times \ A_{663} \quad (2)$$

$$\text{Total chlorophyll} = (Chl\ a + Chl\ b) \quad (3)$$

$$\text{Total carotenoids}(\mathrm{mg\ g^{-1}\ FW}) = 4.2 \times \ A_{452.5}{-}[(0.0264 \times Chl\ a) + (0.426 \times Chl\ b)] \quad (4)$$

FW = Fresh weight
A_{663} = Absorption at 663 nm
A_{644} = Absorption at 644 nm
$A_{452.5}$ = Absorption at 452.5 nm

Membrane stability index (MSI) was measured in different salt treated plants following the method proposed by Pinhero and Fletcher (1994). Fresh leaf samples (1 g) were crushed and placed in 250 mL flask containing 100 mL deionized DDW. The flask mouth was sealed and placed in the water bath maintained at 32 °C. After 2 h, the electrical conductivity of the medium (EC_1) was measured. Then, the samples were autoclaved (121 °C, 30 min) to complete tissue degradation and release of all the electrolytes. Samples were cooled to 25 °C and final EC_2 measured. The MSI was calculated with formula:

$$\mathrm{MSI}(\%) = (1{-}EC_1/EC_2) \times 100 \quad (5)$$

Proline content in paddy plants was determined by the method of Bates et al. (1973). Fresh leaves (0.5 g) were homogenized in 3.00 mL of 5% (w/v) sulfosalicylic acid. The homogenate was centrifuged (10,000 × *g*, 10 min) at 4 °C (Remi CPR-30 Plus). Supernatant (500 µL) was treated with ninhydrin and glacial acetic acid (1:1, v/v). The mixture was boiled for 30 min at 100 °C, and then the reaction was terminated on ice for 5 min. The reaction mixture was extracted with equal volume of toluene. The chromophore containing toluene was aspirated from the upper aqueous phase, warmed at room temperature and absorbance read at 520 nm by (Evolution 201 UV–Vis spectrophotometer). Proline content was determined by comparing with the standard using L-proline (Sigma-Aldrich USA), and calculated on a fresh weight basis (µ mole g^{-1} FW).

$$\mu\text{mole proline/g FW} = [(\mu\text{g proline/mL} \ \times \ \text{mL toluene})/115\ \mu\text{g/mole}] / [(\text{g sample})/5] \quad (6)$$

Na^+ and K^+ were determined with flame photometer (Systronics-130) after wet digestion of paddy plant (dried) materials with HNO_3-$HClO_4$ (3:1). The dried crust of plant material (1.00 g) was digested with 10.00 mL of digestion mixture and kept overnight. After digestion, the flaks containing plant samples were placed on a hot plate and heated until the brown fumes turned white. On cooling, the digested samples were diluted with 50.00 mL of doubled distilled water and filtered through Whatman filter paper No 42. The filtrate was used for estimation of Na^+ and K^+.

2.6.1. Antioxidant enzymatic activities

After effective salt treatment, paddy plant leaves were sampled for the analyses of different enzymatic activities. About 0.2 g of fresh paddy leaves was homogenised in 2.00 mL of ice cold phosphate buffer (pH 7.8) containing 1 mM EDTA with sterilized mortar and pestle. The homogenate was centrifuged (10,000 rpm, 15 min) at 4 °C. The supernatant was used for enzyme assays according to Wang et al. (2012). The specific enzyme activity for all enzymes is expressed as unit mg^{-1}.

Catalase (CAT) (EC 1.11.1.6) activity was assayed by monitoring the decomposition of H_2O_2 at 240 nm (Aebi, 1984). The reaction mixture (3 mL) consisted of 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 0.1% H_2O_2, and 0.1 mL of enzyme extract. The reaction was initiated by adding the enzyme extract. The decrease in H_2O_2 levels was determined by measuring the absorbance at 240 nm with spectrophotometer, and quantified using extinction coefficient (36 mM^{-1} cm^{-1}).

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was examined measuring its effectiveness in inhibiting the photoreduction of nitroblue-tetrazolium (NBT) as described by Giannopolitis and Ries (1977). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 130 mM methionine, 0.75 mM NBT, 0.02 mM riboflavin and 0.1 mL enzyme extract. Riboflavin was added as the last component, and reaction initiated by placing the tubes under two 20 W fluorescent lamps, which lasted for 10 min. Non-illuminated and illuminated reactions without enzyme extract served as calibration standards. The absorbance of the reaction mixture and the blank control was measured at 560 nm. One unit of SOD activity (U) is defined as the amount of enzyme required to cause 50% inhibition of the NBT photoreduction rate, and the results are expressed as unit mg^{-1} protein of FW. One unit of SOD is defined as the amount of enzyme that inhibits 50% NBT photo-reduction.

Peroxidase (POX) (EC 1.11.1.7) activity was based on the oxidation of Guaiacol using hydrogen peroxide (Zhang et al., 1996). The reaction was initiated by adding 20 µL of the enzyme extract to 3 mL of reaction mixture of 100 mM phosphate buffer (pH 7.0), Guaiacol solution (20 µL) and 10 µL of hydrogen peroxide solution. The absorbance was measured at 470 nm at time points of reaction initiation and 5 min later with spectrophotometer. Enzyme activity was quantified based on the amount of tetra-guaiacol formed using the extinction coefficient (26.6 mM^{-1} cm^{-1}). One unit of peroxidase activity is expressed as µmoL mL^{-1} H_2O_2 decomposed per min.

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was assayed according to Nakano and Asada (1981). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.1 mL enzyme extract. The reaction was initiated by addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 3 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}). One unit of APX is defined as 1 mM mL^{-1} ascorbate oxidized per min.

Protein concentration in paddy plant was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard.

2.7. Statistical analyses

The data are expressed as the means of the 3 replicates ± SE. One-way analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences between the means of treatments according to Duncan's multiple range test (P = 0.05). The relationships of paddy plant growth parameters and antioxidant enzymes for all data from the treatments were examined by Pearson's correlation analysis. All statistical analyses were performed using SPSS (Version 20: IBM, Armonk, NY, USA).

Table 1
Promising soil bacterial isolates with different plant growth promoting activities.

Determinants	Nitrogen fixation	Phosphate solubilization	Siderophore production	HCN production	IAA production	EPS production	ACC deaminase
SRV1	+	−	−	−	+	+	−
SRV2	+	−	−	+	+	−	−
SRV3	+	+	−	−	+	−	−
SRV4	+	−	−	+	+	+	+
SRV5	−	−	+	+	+	+	−
SRV6	−	−	+	+	+	−	−
SRV7	+	−	−	+	+	−	−
SRV8	−	+	−	−	+	+	−
SRV9	−	+	−	−	+	+	−
SRV10	−	+	−	+	+	+	−
SRV11	+	−	−	−	+	−	−
SRV12	+	−	+	−	+	−	−
SRV13	−	+	+	−	+	−	−
SRV14	−	−	+	+	+	−	−
SRV15	−	+	−	−	+	−	−
SRV16	+	−	+	−	+	+	−
SRV17	+	+	+	−	+	−	−
SRV18	−	+	−	+	+	+	−
SRV19	+	−	−	−	+	+	−
SRV20	+	−	−	+	−	+	−
SRV21	+	+	−	+	+	−	−
SRV22	−	+	−	+	+	−	−
SRV23	+	−	−	−	+	+	−
SRV24	−	−	+	+	−	−	−
SRV25	−	−	+	−	−	−	−
SRV26	+	−	+	+	+	+	−
SRV27	+	−	−	−	+	+	−
SRV28	−	+	+	+	+	−	−
SRV29	−	−	+	+	+	−	−

HCN = Hydrogen cyanide, IAA = Indole acidic acid, EPS = Exopolysaccharides, ACC = 1-aminocyclopropane-1-carboxylate.

3. Results

3.1. Isolation, PGPR attributes and molecular characterization of bacterial isolate

In this study, about 110 rhizobacterial strains with different morphology were isolated from saline soils of semiarid regions of Sandila situated at Hardoi districts of Uttar Pradesh, India. About 29 promising bacterial strains having at least one PGPR traits viz N_2-fixation, P-solubilization, HCN, siderophore, EPS, IAA, and ACC deaminase activities were screened (Table 1). All 29 strains were evaluated at different doses NaCl concentrations (0–1800 mM). Only one bacterial strain (Gram positive) showed positive results for the N_2-fixation, EPS, IAA ($16.1 \pm 0.5\,\mu g\,mL^{-1}$), HCN production and ACC deaminase (296 ± 11.2 nmol α-KB mg^{-1} protein h^{-1}) activity. Molecular characterization, based on the nucleotide BLAST (BLASTn) analysis of 16S rRNA sequence indicated that the bacterial isolate has closest relationship with *C. albidum* with 98% similarity, and named as SRV4 strain. To further confirm the results of BLASTn, phylogenetic analysis of SRV4 strain was performed with some other species of *Curtobacterium* and a strain of *C. albidum* DSM20512. Results revealed its relatedness with several other strains of *Curtobacterium* sp. (Fig. 1). The sequence data of *C. albidum* SRV4 has been deposited to the GenBank nucleotide sequence database with the accession number KX 81071.

3.2. Effect of isolated bacterial strain on paddy plant growth parameters

The results related to different saline regimes on paddy plant growth parameters in presence and absence of SRV4 strain has been depicted in Table 2. The addition of salt (NaCl stress) disturbed paddy growth performance. Across different NaCl treatments, highest reduction in paddy plant growth parameters was noted at 300 mM NaCl, followed by 200 mM and 100 mM NaCl compared to control (without NaCl treatment). But, inoculation of SRV4 isolate showed enhancement (8.25–33.2%) in paddy plant growth parameters and variations due to treatments were significant ($P < 0.001$–0.005). Inoculation of SRV4 strain along with different doses of NaCl treatments to the paddy plants enhanced plant height (from 10.37 to 19.01%), root length (from 11.72 to 23.51%), tiller number (from 8.25 to 33.2%) and dry wt. (from 8.65 to 22.89%) compared to sets treated with respective doses of NaCl only.

3.3. Effect of bacterial strain on chlorophyll pigments, osmolytes and antioxidant enzymes

The selected photosynthetic pigments of paddy plants varied significantly ($P < 0.001$) due to various treatments (Table 3). When the paddy plant was treated with different doses of NaCl (100 mM to 300 mM NaCl concentration) about 13.63–44.81% reduction in MSI was noted (Fig. 2a). However, SRV4 treated sets exhibited MSI enhancement by 23.31% (100 mM), 29.36% (200 mM) and 41.91% (300 mM) NaCl concentration compared to respective controls (only NaCl treatment). Across different NaCl doses alone, or in combination with SRV4, proline content in paddy plants enhanced significantly and ranged from 2.12 to 4.07 μ mole g^{-1} fresh weight (Fig. 2b). The addition of SRV4 strain indicated further enhancement in proline content ranging from 7.38 to 11.11%. The increased accumulations of proline content in paddy plants due to application of strain SRV4 proved its efficacy to induce systemic tolerance in paddy plants against salt stress.

Fig. 3a shows elevation in Na^+ accumulation by paddy plants (1.11–2.03 mg g^{-1} FW) from 100 to 300 mM. But the application of respective NaCl doses with SRV4 strains reduced Na^+ accumulation (9.0–27.09%). NaCl treatments declined K^+ in paddy plants (Fig. 3b) or could disturb cytoplasmic Na^+/K^+ homeostasis. However, SRV4 isolate application with different doses of NaCl enhanced K^+ accumulation (ranged from 11.22 to 39.21%). The CAT was enhanced with the increasing NaCl dose (Fig. 4a). The increase in CAT activity across different NaCl doses ranged from 21.10 to 44.95%. Inoculation of SRV4 with different NaCl doses further enhanced CAT activity (ranging from 27.27 to 51.26%) compared to respective controls. Similar trends for the enhancement in SOD (Fig. 4b), POX (Fig. 4c) and APX activity

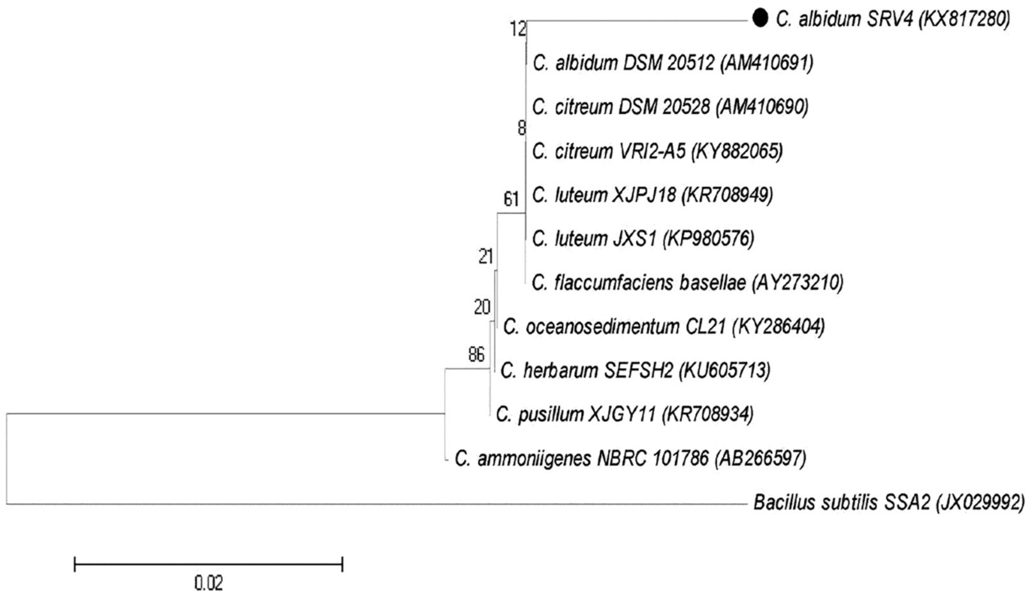

Fig. 1. Phylogenetic relationship of isolated rhizobacterial strain SRV4 with other closely related bacterial strains based on 16s rRNA gene sequence available from National Center for Biotechnology Information (NCBI) database library. (The branching pattern was generated by neighbour-joining method from the Molecular Evolutionary Genetics Analysis (MEGA) version 6 software package (Tamura et al., 2013), using *Bacillus subtilis* SSA2 as an out group member).

Table 2
Effect of *Curtobacterium albidum* SRV4 on paddy plant morphology under NaCl treatments. The values given are means of 3 independent experiments ± SE. N = 24 (8 treatments × 3 replicates).

Treatments	Plant height (cm)	Root length (cm)	Tillers (plant^{-1})	Plant dry weight (g plant^{-1})
Control	55.36 ± 1.27^{a}	18.56 ± 1.24^{a}	5.33 ± 0.66^{a}	18.16 ± 1.41^{a}
Control + SRV4	68.20 ± 1.58^{b}	23.26 ± 1.63^{b}	7.33 ± 0.33^{b}	23.03 ± 1.54^{b}
100 mM	52.60 ± 1.92^{a}	16.03 ± 1.17^{c}	5.02 ± 0.57^{a}	16.73 ± 1.88^{c}
100 mM + SRV4	62.60 ± 1.93^{c}	19.8 ± 1.24^{d}	6.66 ± 0.33^{c}	20.56 ± 1.49^{d}
200 mM	47.26 ± 1.43^{d}	13.24 ± 1.00^{ab}	4.66 ± 0.33^{d}	14.46 ± 1.06^{ab}
200 mM + SRV4	54.83 ± 1.76^{a}	15.86 ± 1.41^{ac}	5.66 ± 0.88^{a}	17.3 ± 1.36^{ac}
300 mM	41.16 ± 1.28^{ab}	10.80 ± 1.15^{ad}	4.0 ± 0.57^{ab}	10.4 ± 1.76^{ad}
300 mM + SRV4	45.43 ± 1.39^{ac}	12.06 ± 1.23^{ba}	4.33 ± 0.33^{d}	11.3 ± 1.08^{ba}
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.005$	$P < 0.001$

Different letters indicate significant difference between treatments for each parameter ($P < 0.05$).

Table 3
Effect of NaCl stress and *Curtobacterium albidum* SRV4 on chlorophyll contents in paddy plants. The values given are means of 3 independent experiments ± SE. N = 24 (8 treatments × 3 replicates).

Treatments	*Chl* a (mg g^{-1} FW)	*Chl* b (mg g^{-1} FW)	Total *Chl* (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Control	1.73 ± 0.01^{a}	3.48 ± 0.06^{a}	5.21 ± 0.06^{a}	0.88 ± 0.02^{a}
Control + SRV4	2.07 ± 0.03^{b}	4.76 ± 0.02^{b}	6.83 ± 0.01^{b}	0.98 ± 0.01^{b}
100 mM	1.32 ± 0.02^{a}	3.19 ± 0.20^{c}	4.51 ± 0.19^{c}	0.72 ± 0.07^{c}
100 mM + SRV4	1.53 ± 0.04^{c}	3.83 ± 0.09^{a}	5.37 ± 0.08^{a}	0.94 ± 0.08^{b}
200 mM	1.09 ± 0.05^{ab}	2.80 ± 0.01^{ab}	3.90 ± 0.04^{ab}	0.66 ± 0.03^{ab}
200 mM + SRV4	1.23 ± 0.01^{d}	3.28 ± 0.15^{a}	4.51 ± 0.14^{c}	0.77 ± 0.02^{c}
300 mM	0.82 ± 0.08^{cb}	2.26 ± 0.07^{cb}	3.08 ± 0.14^{cb}	0.49 ± 0.02^{d}
300 mM + SRV4	0.89 ± 0.08^{cb}	2.56 ± 0.02^{cb}	3.46 ± 0.06^{d}	0.53 ± 0.01^{cb}
Significance level	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Different letters indicate significant difference between treatments for each parameter ($P < 0.05$). FW = Fresh weight.

(Fig. 4d) was also noted for different doses of NaCl alone or in combination with SRV4 compared to respective controls. The Pearson's correlation analysis showed negative relationship between paddy plant growth parameters and antioxidant enzymes across treatments (Table 4).

4. Discussion

In this investigation, a comprehensive screening of native bacterial population from saline soils was carried out for their plant growth promoting attributes and ability to survive at different salt concentrations. The higher EC value (6.8 dS m^{-1}) of the collected soil samples indicated saline nature of the soil. Therefore, the isolated bacterial population, having ability to grow and survive in saline soils, may be considered as salt-tolerant. Out of 29 PGPRs, the most potent SRV4 isolate having salinity tolerance (up to 1500 mM NaCl) and plant growth promoting attributes, was selected for inoculation to the paddy plants.

High soil salinity has been considered as most determinant adverse and stress condition, affecting paddy crop productivity by affecting plant physiology and growth parameters (Upadhyay et al., 2011). However, the recent investigations suggest that inoculation of salt-tolerant microbial agents to saline soils improved crop plant growth by alleviating the salt stress (Singh et al., 2016; Vimal et al., 2017). Therefore, present study investigated the influence of an efficient salt-tolerant rhizobacterium *C. albidum* SRV4 application on plant growth

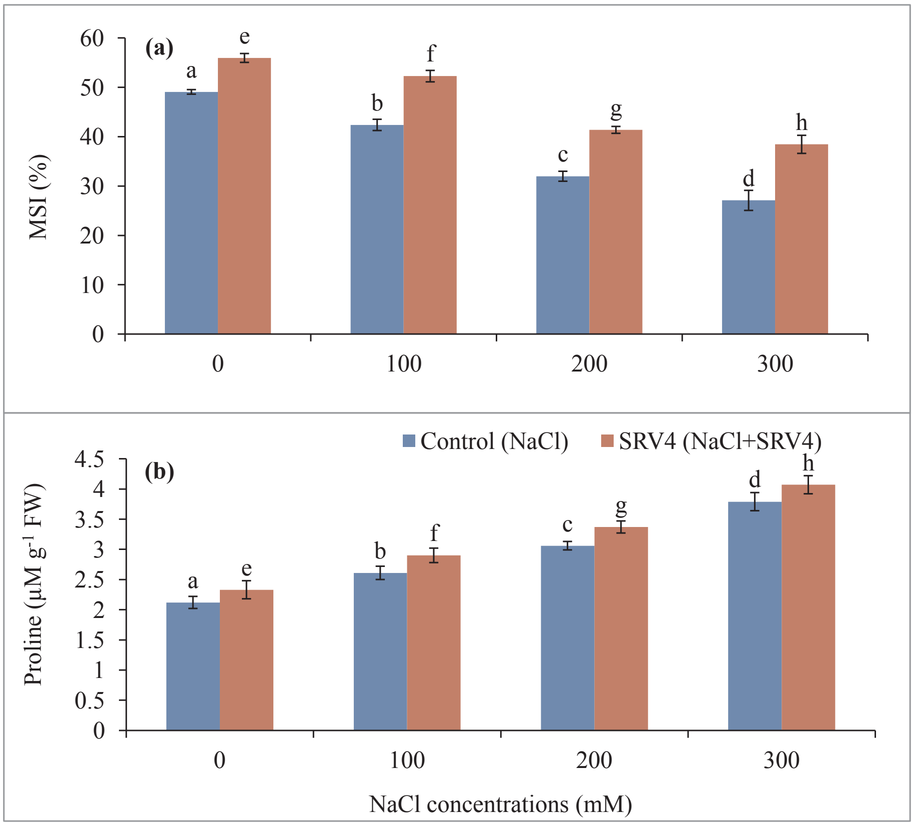

Fig. 2. Effect of *Curtobacterium albidum* SRV4 on (a) Membrane stability index (MSI) (b) Proline contents paddy plants. The vertical line on each bar represents means of 3 independent experiments ± SE. Different letters on each bars indicate significant difference at $P < 0.05$ level.

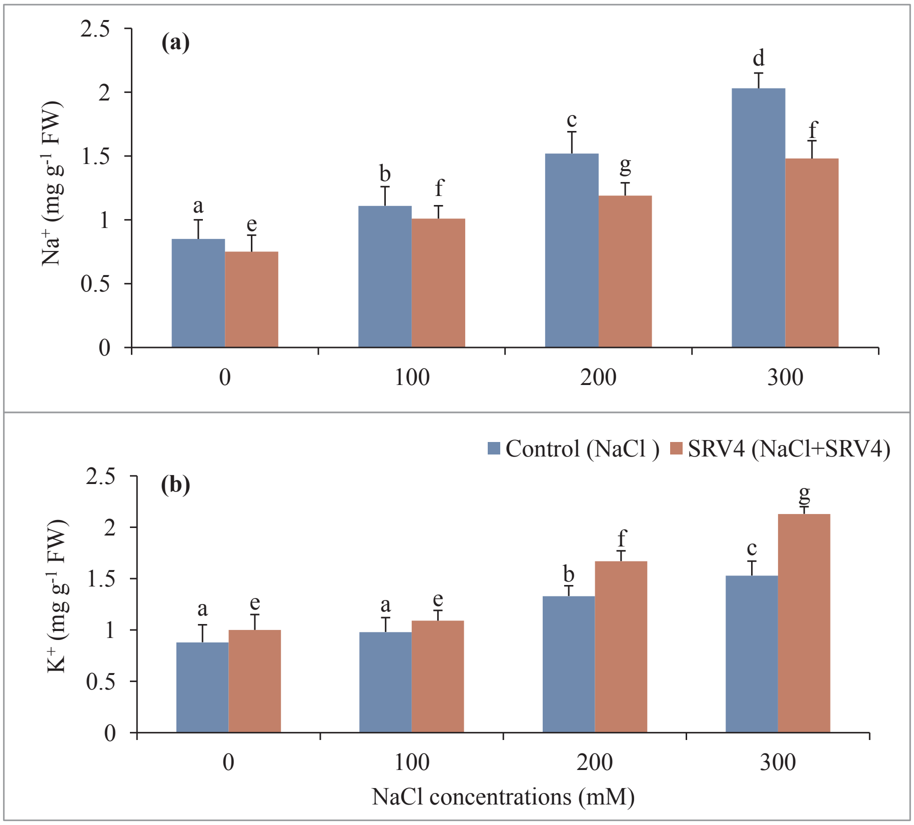

Fig. 3. Effect of different salinity concentrations on (a) Na^+ and (b) K^+ uptake by paddy plants. The vertical line on each bar represents means of 3 independent experiments ± SE. Different letters on each bars indicate significant difference at $P < 0.05$ level. FW = Fresh Weight.

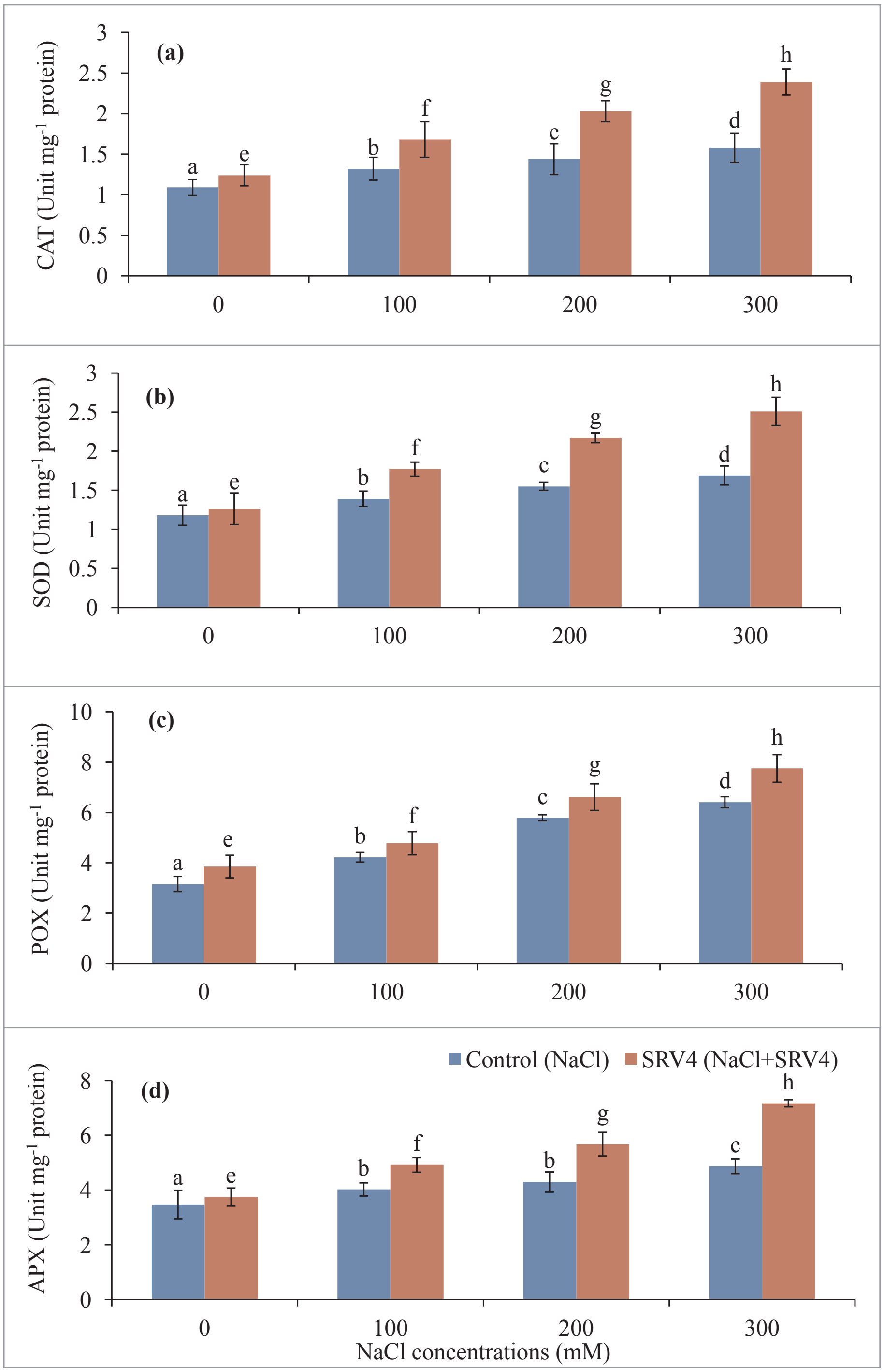

Fig. 4. Impact of *Curtobacterium albidum* SRV4 inoculation on antioxidative enzymatic activities (a) catalase activity (CAT), (b) superoxide dismutase activity (SOD), (c) peroxidase (POX) and (d) ascorbate peroxidase (APX), in paddy plants under salinity stress. The vertical line on each bar represents means of 3 independent experiments ± SE. Different letters on each bars indicate significant difference at $P < 0.05$ level.

parameters, osmolytes and antioxidant enzymes levels of paddy under various salinity levels.

Inoculations of PGPRs to the soils have improved crop plant growth by their ability to solubilize phosphate, production of various low-molecular mass compounds (phytohormones) and/or antioxidants, accumulation of osmoprotectants such as proline, and balancing the Na^+/K^+ ratio (Upadhyay et al., 2011; Upadhyay and Singh, 2015). Salt stress due to Na^+ accumulation can induce nutrient and osmotic imbalance in plants that can lead to stunted paddy plant growth (Khan et al., 2016). The data of present study showed that accumulation of Na^+ in paddy plants declined when the crop was grown with the inoculation of SRV4 bacterium. An improved EPS production by SRV4 strain could be one the major reasons to help paddy plants to overcome salinity stress by reducing the supply of Na^+ ions to plants (Upadhyay et al., 2011). Further, the inoculation of SRV4 indicated an elevated level of K^+ accumulation in the paddy plants, might be the additional reason in the mitigation of salt stress imposed by high salinity. It is reported that plant survives excessive salt concentrations through Na^+ omission, osmotic stress tolerance, and tissue Na^+ adaptation (Munns and Tester, 2008). It is assumed that in paddy plants, Na^+/K^+

Table 4
Pearson's correlation (2-tailed) between plant growth parameters and antioxidant levels in paddy plant treated with *Curtobacterium albidum* SRV4 under NaCl stress conditions. N = 24 (8 treatments × 3 replicates).

Parameters	Plant height	Root length	Tiller number	Plant dry weight	CAT	SOD	POX
Root length (cm)	0.865^{**}						
Tiller number (plant^{-1})	0.774^{**}	0.663^{**}					
Plant dry weight (g plant^{-1})	0.848^{**}	0.760^{**}	0.716^{**}				
CAT (Unit mg^{-1} protein)	-0.380^{NS}	-0.510^{*}	-0.351^{NS}	-0.458^{*}			
SOD (Unit mg^{-1} protein)	-0.385^{NS}	-0.466^{*}	-0.385^{NS}	-0.501^{*}	0.866^{**}		
POX (Unit mg^{-1} protein)	-0.558^{**}	-0.599^{**}	-0.455^{*}	-0.687^{**}	0.722^{**}	0.815^{**}	
APX (Unit mg^{-1} protein)	-0.368^{NS}	-0.422^{*}	-0.172^{NS}	-0.513^{*}	0.782^{**}	0.875^{**}	0.781^{**}

NS = Not significant; * Significant at $P < 0.05$; ** Significant at $P < 0.01$ level.

homeostasis was well controlled due to inoculation of SRV4 strain. Several reports also demonstrated that a PGPRs inoculation manages the K^{+}/Na^{+} ratio and therefore, salinity tolerance in crop plants (Nadeem et al., 2013). A statistically significant enhancement in paddy plant growth parameters following co-inoculation with SRV4 bacterium (Table 2), compared to un-inoculated controls indicates that this bacterium, like other PGPRs (Upadhyay et al., 2011), also has the potential to control deleterious effects of salinity stress on growth of paddy plants.

The photosynthetic pigments such as chlorophyll a, b, total-chlorophyll and carotenoids decreased significantly ($P < 0.001$) in the paddy plants at higher doses of NaCl treatments. But due to inculcation of SRV4 strain, a significant increase in photosynthetic pigments was noted. The decrease in photosynthetic pigment contents at higher NaCl doses may be due to salinity stress developed by higher concentrations of Na^{+}. A higher dose of NaCl treatment has been noted to decline the photosynthetic pigments (chlorophylls and carotenoids) status. Decline in photosynthetic pigments was maximum at 300 mM NaCl. However, application of SRV4 strain with different doses of NaCl showed efficiency of pigments restoration compared to NaCl treatments alone. The restoration of pigments shows the ability of SRV4 strain to induce systemic tolerance under NaCl stress. Excess amount of Na^{+} as well as Cl^{-} may disturb the ionic equilibrium of the cell, leading to damage of membrane stability. The higher carotenoids (non-enzymatic antioxidants) generation due to addition of SRV4, can serve as the accessory pigment to protect the various chlorophyll pigments under salinity stress. The higher proline content generation in response to elevated salinity, has also been reported as the powerful antioxidant in managing oxidative adjustments and protects intracellular macromolecules against dehydration (Upadhyay and Singh, 2015). An improved production of various photosynthetic pigments in PGPRs inoculated paddy seedlings under salt stress as reported by Kumar et al. (2017) supports the findings of this investigation.

As the data of present study indicated that the antioxidative enzymatic activities such as CAT, SOD, POX and APX increased in paddy plants with increase in NaCl dose. Plants have developed defense enzymatic systems to alleviate and re-nature the damages caused by salinity mediated oxidative stress (Upadhyay et al., 2012). In general, the increase in the antioxidative enzyme activities in response to stress conditions such as fly ash (FA) treated soil of paddy fields was found to be directly correlated with the levels of heavy metals accumulation in the leaves of paddy plants (Singh and Pandey, 2013). In the present experiments, an enhanced antioxidative enzymes production in paddy plants, possibly could be to neutralize the adverse impact of higher salinity stress and is in conformity with the results of Gill and Tuteja (2010) who demonstrated higher antioxidant ability of plants under abiotic stress. The negative correlations between plant growth parameters and antioxidant enzymes levels in the present investigation indicated that inoculation of salt tolerant *C. albidum* SRV4 to soil reduces the salinity stress in paddy plants. These results suggest that higher antioxidative enzyme requirement by paddy plant at higher salinity levels, could play as important role to protect plants against oxidative injury.

5. Conclusions

The aim of the present investigation was just a preliminary study to isolate and identify the efficient PGPR strains from the saline soils that could be evaluated from pot to field conditions to improve the growth and yield of paddy crop under salinity stress. The pots inoculated with SRV4 strain increased paddy plant growth compared to the non-inoculated controls under various doses of salt concentrations. Based on the above results, this study suggests that inoculation of *C. albidum* SRV4 had significant impact on paddy growth due to their plant growth promoting attribute under salinity stress. A negative correlation between paddy plant growth parameters (inoculated with *C. albidum* SRV4) and antioxidative enzymatic activities confirmed that an adverse impact of higher dose of NaCl on paddy plant is counteracted by salt tolerant inoculated bacteria. The data showed that salinity toxicity at higher doses of NaCl may cause harmful osmotic disturbance in the paddy plants, and osmolytes and antioxidative enzymes could play a defensive role against damages due to salinity stresses. To the best of our knowledge, this may be the first report with reference to the *C. albidum* inoculation in soils to alleviate salinity stress and improve paddy yield. With reference to the combined effect of microbial inoculants and their survival under salinity stress and their survival under salinity stress and their competition with other indigenous microbial flora, it would be more effective and viable to apply the locally well adapted salt tolerant native microbial agents like *C. albidum* SRV4 to ensure the best results on paddy crop productivity under salinity stress.

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Effect of salt tolerant *Bacillus* sp. and *Pseudomonas* sp. on wheat (*Triticum aestivum* L.) growth under soil salinity: A comparative study

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Abstract

This study was conducted to examine the comparative effect on wheat plant health inoculated with the two different rhizobacterial strains *Bacillus* sp. (JG3) and *Pseudomonas* sp. (JG7) under soil salinity. Total seven potential salt tolerant strains were isolated from the saline soils of BBAU-Lucknow. The bacterial strains have been investigated for nitrogen fixatation, phosphate solubilization, ammonia, indole acetic acid and hydrogen cyanide production activities. Based on morphological and biochemical activities the strains JG3 was designated as *Bacillus* sp. and the strain JG7 was designated as *Pseudomonas* sp. Both the strains witness positive for the different plant growth promoting traits. In comparison of strain JG7, strain JG3 inoculated wheat seeds enhance plant height by 32.32%, root length by 37.84%, fresh weight by 28.2% and dry weight by 15.51% in FYM amended soils. We observe in this study that seeds treated with *Bacillus* sp. found significantly effective in plant growth promotion compared to *Pseudomonas* sp. in saline soil. Based on the comparative experimental study reported herein, it is pointedly observed that the use of salt tolerant PGPRs are effective for facilitating plant health in salt stress environments.

Introduction

Salinity is one of the most common environmental stress factors that adversely affect plant growth and crop production in cultivated areas worldwide.[1,2] Primary saline conditions appear naturally in environment yet anthropogenic activities are responsible for secondary salinity. Increased urbanization and deforestation are two important human derived activities for salinity. A number of reports are on soil salinization and their influences on crop productivity, land degradation and ecological disturbances are reported worldwide. Excess amount of salts effected physical chemical as well as the biological properties of soils. Plant health in saline soils is considerably decline owing to poor nutrition, osmotic stress and reduced microbial diversity.[3]

Wheat is major cereal crops in India and mainly cultivated in rain-fed areas. Salinity is a major constraint, which hampers wheat production, causing a loss of about 65% in yield in moderately saline soils.[4] Salt stress inhibits photosynthesis, protein synthesis, and other metabolic processes in plants.[2] The development of salt stress tolerant varieties through genetic engineering and plant breeding technology is often innovative technology but a long drawn process taking months to years for successful development. Microbial technology in agriculture is one the needed technology at present and future for sustainable crop productions.[5]

Since the elaboration of rhizospheric concept by Hilter in 1904 various rhizospheric microorganisms from different groups have been reported for their plant health promoting activities. The plant growth promoting rhizobacteria not only encourage plant health but emerged as important component of salt stress management.[6,7] The different PGPRs *Bacillus, Pseudomonas, Azospirillum, Agrobacterium, Achromobacter, Serratia,* have been reported for their PGP activity under different ecological conditions. Among these genus Bacillus the Gram positive and Gram Negative Pseudomonas are most extensively studies rhizobacteria facilitating plant health. *B. amyloliquefaciens, B. licheniformis, B. megaterium, B. pumilus*, and *B. subtilis,* are some important member of genus Bacillus and reported for plant growth and stress managements.[8,9] An increased agricultural production in response to *Pseudomaonas* inoculation has been reported through different mechanism.[10] Application of salt tolerant PGPR can be beneficial technology for wheat cultivation in saline areas. PGPR colonise roots and enhances root health through proving nutrient to plants and in return plant provide exudates to PGPR. At present there is dire need of fruitful sustainable agricultural technology in climate change scenarios.

The objectives of this study are: i) Isolation and purification of the rhizobacterial strains from saline environment; ii) Efficacy of salt tolerant potential of isolated rhizobacterial strains; iii) Assays biochemical and plant growth promoting traits of salt tolerant strains; iv) *In vitro* study on potential of selected isolates from *Triticum aestivum* under soil salinity.

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Key words: Rhizobacteria; *Bacillus* sp.; *Pseudomonas* sp.; Soil salinity; Plant growth.

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Contributions: SRV designed the experiment and compiled whole manuscript. JG performed experiment. JSS analyzed data, refined scientific languages and guided experiment.

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Materials and Methods

Collection of soil samples

Soil sample were collected from the rhizosphere of grasses growing on non-fertile saline soils form the premises of Babasaheb Bhimrao Ambedkar University, Lucknow (Figure 1).

Isolation of rhizobacterial strains

1 gram of rhizospheric soil was diluted in 9 mL of MQ water and serially diluted up to 10-6 and spread on nutrient agar media. Plates were incubated for 24 hours at 28±2°C and development of colonies was observed. The colonies were purified and preserved in nutrient agar slants at 4°C.

Rhizobacterial salt tolerance efficacy

All the isolates were examined for NaCl tolerance capacity (up to 1000 mM) in nutrient broth as well as streaked on NA plates. In broth condition growth was monitored with UV-Vis double beam spectrophotometer at 610 nm.

Phenotypic characterization

Phenotypic characterization of all bacterial isolates was done according to *Bergey's Manual of Systematic Bacteriology* 2010 (Table 1).

Gram's staining

Late log phase culture of isolated strains were smeared on glass slides and fixed under Bunsen burner. The staining of all isolates were done according to Coico (2005)[11] and observed under phase contrast microscope.

Biochemical tests

Citrate utilization test

Citrate utilization test were done according to Koser (1924).[12] Freshly grown bacterial culture were streaked on Simmon Citrate agar plate and incubated for 24 hours for 28±2°C.

Amylase production test

Amylase production test were done according to Palleroni and Holmes (1981).[13] Spot inoculation was done on starch agar medium and was incubated for 24 hours at 28±2°C. After 24 hours the plate was flooded by iodine solution. A transparent zone around colonies appears for positive result.

Catalase test

The catalase activity was done according to Graham and Parker (1964).[14] Smear of bacterial culture was made on clean slide with help of inoculating loop. On pouring few drops of 3% hydrogen peroxide, bubbles of oxygen were observed on the slide.

Casein hydrolysis

Casein agar hydrolysis was performed with the method of Seeley and Van Demark (1970).15 Skimmed milk agar media were prepared and bacterial strains were spot inoculated on the plates and incubated for 24-48 hour at 28±2°C.

Carbohydrate utilization assays

Twelve carbohydrate utilization tests were examine with carbohydrate utilization kit (Himedia)-Mumbai (KB-009A) India.

Plant growth promoting test of isolated rhizobacterial strains

Phosphate solubilization

Phosphate solubilization test were performed according to Pikovaskaya (1948).[16] Isolated strains were spot inoculated on Pikovaskaya agar medium and incubated for 5-7 days at 28±2°C. Phosphate solubilization index (PSI) was calculated by using the formula.

Nitrogen fixation

N2 fixation ability of isolates were analyse on Jenson N agar medium according to Jenson (1954).[17] Appearance of growth on Jenson agar indicates efficiency of N fixation by isolate.

Siderophore production

Siderophore production ability of isolates were determined according to Shwayn and Neilands (1987).[18] Appearance of orange colour zone on cash dye containing plate shows positive iron chelation.

Hydrogen cyanide production

The HCN production test was done according to Lorck (1948).[19] The isolates were subculture on Kings B agar plate supplemented with glycerol (15 mL L^{-1}). The filter paper was dip in Picrate/Na_2CO_3 solution and placed on upper lid of Petri plate and incubated at 28±2°C after sealing. Colour changes from yellow to orange, red, brown, or reddish brown was recorded as an indication of weak, moderate, or strongly cyanogenic potential, respectively.

Ammonia production

The NH3 production of isolated bacterial strains was done according to method of Cappucino and Sherman (1992).[20] Peptone water broth was prepared in test tubes and was inoculated by bacterial strains and incubated for 48-72 hours at 28°C. 1 mL of Nesseler's reagent was poured in test tubes. Change in colour from yellow to orange was observed.

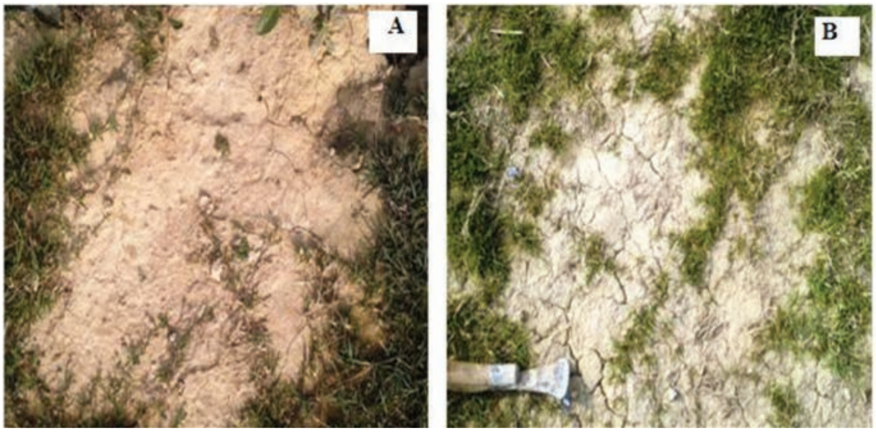

Figure 1. Photomicrograph of sampling sites of BBAU-Lucknow. (A) Sampling site 1 (B) Sampling site 2.

Table 1. Phenotypic characterization of selected isolates.

Isolates morphology	JG1	JG2	JG3	JG4	JG5	JG6	JG7
Size (cm)	0.1	0.2	0.1	0.1	0.1	0.1	0.1
Form	Circular	Circular	Circular	Circular	Circular	Circular	Circular
Edge	Entire	undulate	Entire	Curled	Entire	Undulate	Entire
Elevation	Crateriform	Flat	Flat	Convex	Flat	Convex	Convex
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	EPS producing
Pigment	No	Yes	Yes	No	Yes	Yes	Yes
Colour	Off white	White	Off white	Light yellow	Orange	Yellow	Pale yellow

Indole acetic acid production

The IAA production test was done according to Gorden and Weber (1951).[21] The strains were inoculated on minimal broth containing different tryptophan concentrations of 50 and 100 µg L^{-1}. The test tubes were incubated for 48-72 hours at 28±2°C. The cultures were centrifuged at 6000 rpm for 10 minutes. About 4 mL freshly prepared Salkowaski reagent is added in 1 mL of supernatant. Appearance of Cherry Red colour is observed for IAA production. For quantification assay optical density was observed at 530 nm in double beam UV visible spectrophotometer.

Antibiotic sensitivity test

The antibiotic sensitivity test was done according to method of Bauer *et al.* (1996).[22,23] This test was performed on Mullar-Hilton agar media plates by placing antibiotic disc of Vancomycin, Kanamycin, Polymyxin-B and Erthromycin. After keeping it for 24-48 hours at 28±2°C zones were formed around the disc were observed.

Experimental design

In vitro complete randomized design pot experiment was performed to study inoculations effect on wheat plant growth. The plastic pots were filled with sterilized soils introduces with primary salinity according to Bharti *et al.* (2013).[24] FYM is used as a carrier in bioformulations yet FYM is also used as organic supplement to soils. The experiment conducted for 4 weeks and irrigation was done with non-saline MQ water. On 29th day the plants were pulled out from pots and analyse for growth parameters.

A. Control (Sterilized soil);
B. Sterilized soil + Farmyard manure;
C. Sterilized soil + Farmyard manure+ Strain JG3 (*Bacillus sp.*);
D. Sterilized soil + Farmyard manure + Strain JG7 (*Pseudomonas sp.*).

Statistical analysis

The collected data were subjected to statistical analysis for analysis of variance (ANOVA) performed with IBM SPSS 20.0. All values are in mean of triplicate ± standard error. The result was considered significant at $p<0.05$

Results

Rhizobacterial isolates

Total seven salt tolerant rhizobacterial

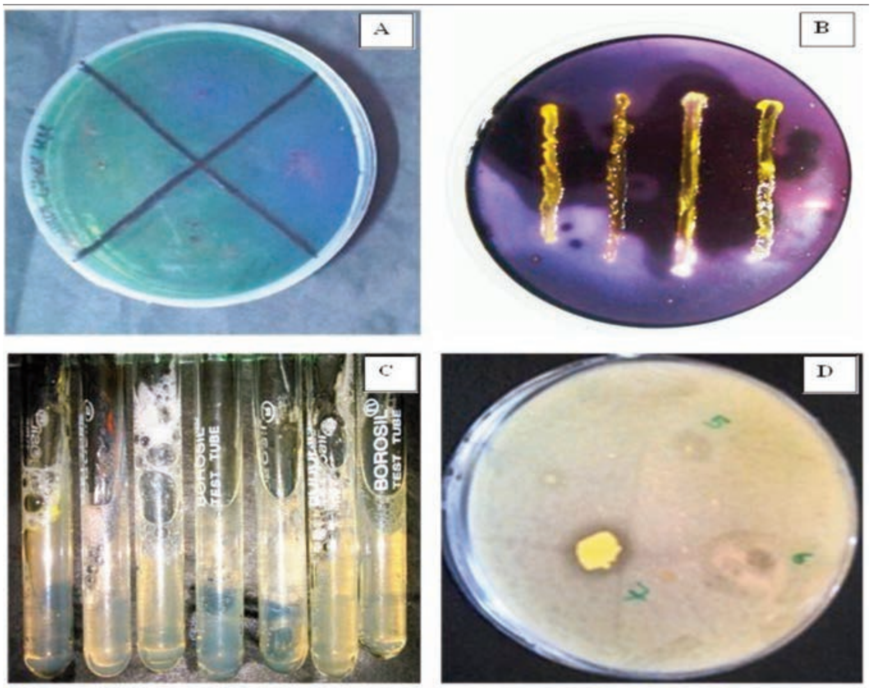

Figure 2. Biochemical activities of isolates. (A) Citrate utilization (B) Amylase production (C) Catalase test (D) Casein hydrolysis.

Figure 3. Development of farmyard based formulation with selected isolates in laboratory. Seed bio-priming.

Table 2. Biochemical activities of isolates rhizo-bacterial strains.

Biochemical activities of isolates	JG-1	JG-2	JG-3	JG-4	JG-5	JG-6	JG-7
Casein hydrolase	-	-	+++	+	+	+	++
Starch hydrolase	-	-	++	-	++	+	-
Catalase activity	++	+	++	-	-	++	++
Citrate utilization	-	+++	–	+++	-	-	++
Gram staining	-	+	+	-	-	+	-

strains with different morphology on nutrient agar medium were isolated and purified for the study (Table 1).

Biochemical test of rhizobacterial isolates

Different biochemical test of all seven isolates were described in (Table 2) and shown in (Figure 2). For citrate utilization capacity of isolates only two isolates JG2 and JG4 were found positive and remaining shows negative result for the test. Amylase production efficiency was shown positive by three strains JG3, JG5 and JG6 and other not found positive. For catalase test, out of 7 bacterial isolates, one isolates (JG3) was excellent positive (+++), two (JG1, JG6) were found moderately positive (++) and two strains (JG2, JG7) were slightly positive and two (JG4, JG5) were found negative (-). Strain JG3 was found excellently positive for casein hydrolysis while JG7 moderate positive, JG4 and JG5 slightly positive and JG1 and JG2 are negative for the test.

Carbohydrate utilization test

Different biochemical test of all seven isolates were given in Table 3.

Plant growth promoting traits of isolates

Plant growth promoting traits of isolates were described in (Table 4) and (Figure 4). IAA production was seen highest by strain JG7 16.3 µgml-1 followed by strain JG3 14.8 µgml^{-1} with amendment of 100 µgL^{-1}L^{-1} of tryptophan.

Development of bio-formulation of selected strains

The bio-formulation of selected best PGPR strains (JG3 and JG7) were done according to procedures of Vidhyasekaran and Muthamilan (1995).23 FYM is used as carrier material for strains (Figure 3).

Wheat cultivars was procured form the local market and were surface sterilized with 70% ethanol followed by 2% Sodium hypochlorite solution for 10 minutes and rinsed in sterile MQ water. Seeds were dipped in sterilized MQ water for control and bacterial suspensions for 10 minutes and dried overnight in laminar.

Comparative study of plant growth parameter

The comparative experiment was shown in Figure 5. FYM amendment with soil enhances plant height by 10.06% while inoculation with strain JG3 enhances plant height by 84.56% and seeds inoculated with JG7 enhance plant height by 48.99% comparative untreated control (Figure 6A). Root length was increased by 15.62% in FYM amended soils, 101.56% in JG3+FYM amended soil, 57.81% in JG7+FYM amended soil compare to control (Figure 6B). Fresh plant weight was highest in JG3 inoculated plants 83.41% followed by 51.25% in JG7 inoculated plants and 14.07% in FYM amended soils (Figure 6C). While wheat plant dry weight was enhance by 94.04% in JG3+FYM amended soil 72.61% in JG7+FYM amended soil and 38.09% in

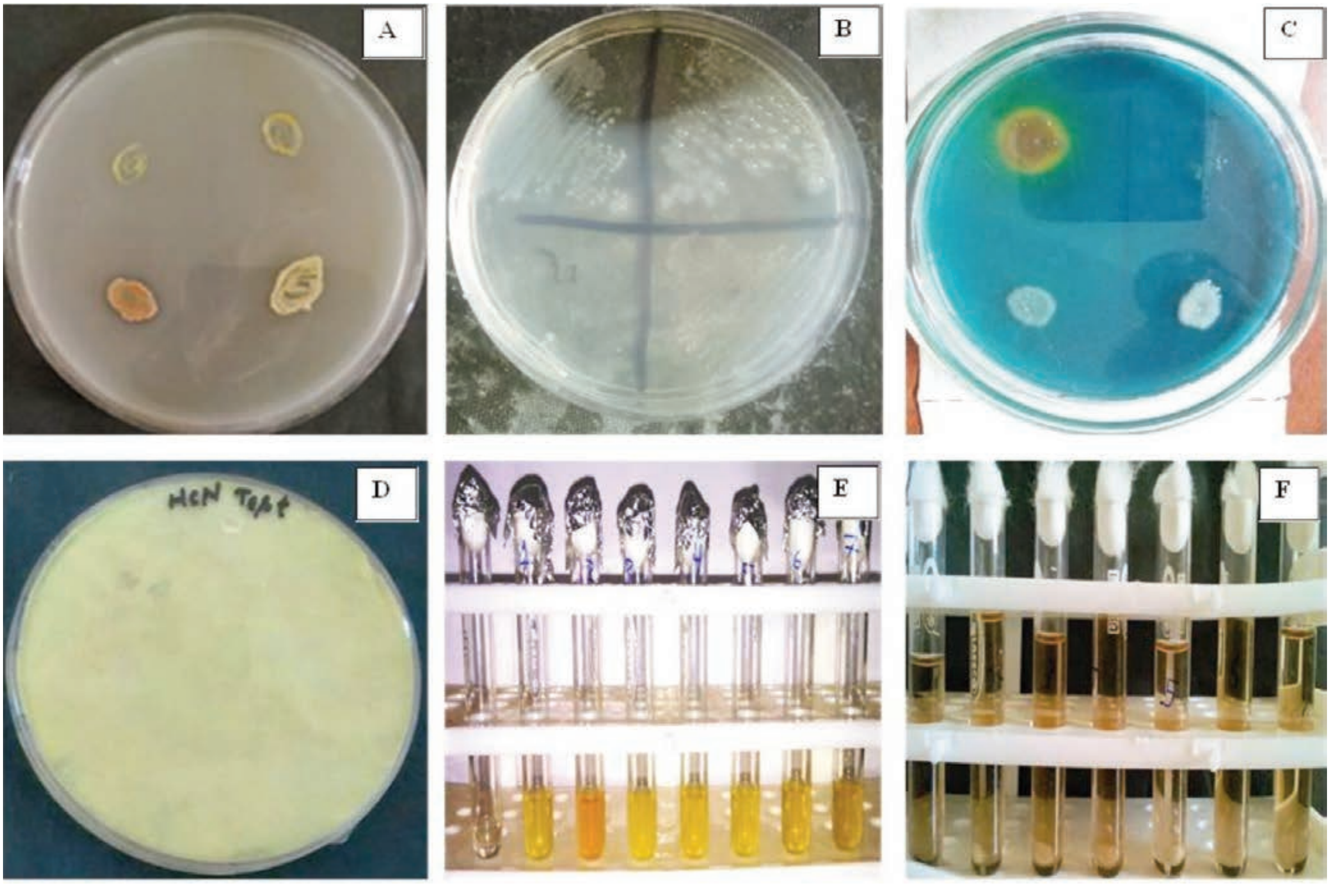

Figure 4. Photomicrograph of plant growth promoting traits of salt tolerant isolates. (A) Phosphate solubilization (B) Nitrogen fixation (C) Siderophore production (D) HCN production (E) Ammonia production (F) IAA production.

FYM treated soil (Figure 6D). Thus in compare to strain JG7 we observe strain JG3 enhances plant height by 32.32%, root length by 37.84%, fresh weight by 28.2% and dry weight by 15.51% compared to FYM amended plants.

Discussion and Conclusions

In the present study seven salt tolerant rhizobacteria were isolated form naturally saline soils and assayed for their plant growth promoting potential and ability to mitigate saline stress of wheat plants. Two best potential isolates on the basis of their morphology and biochemical activities were screened and observed for wheat plant growth promotion under *in vitro* conditions. These isolates were designated as *Bacillus* sp. (Strain JG3) and *Pseudomonas* sp. (Strain JG7). Plant growth promoting rhizobacteria enhance plant health through various known and unknown plant growth promotion mechanisms.[24] Variety of rhizobacterial strains form both group *Bacillus* and *Pseudomonas* are also introduce for plant growth promotion under soil salinity.[25] In this study, *Bacillus* sp. (Strain JG3) and *Pseudomonas* sp. (Strain JG7) were found significant for different PGP traits. Seeds of wheat inoculated with strain JG3 exhibited significant plant growth enhancement than the strain JG7 under primary soil salinity. Wheat plant height, root length, fresh and dry weight was significantly reduced due to salinity stresses. The pres-

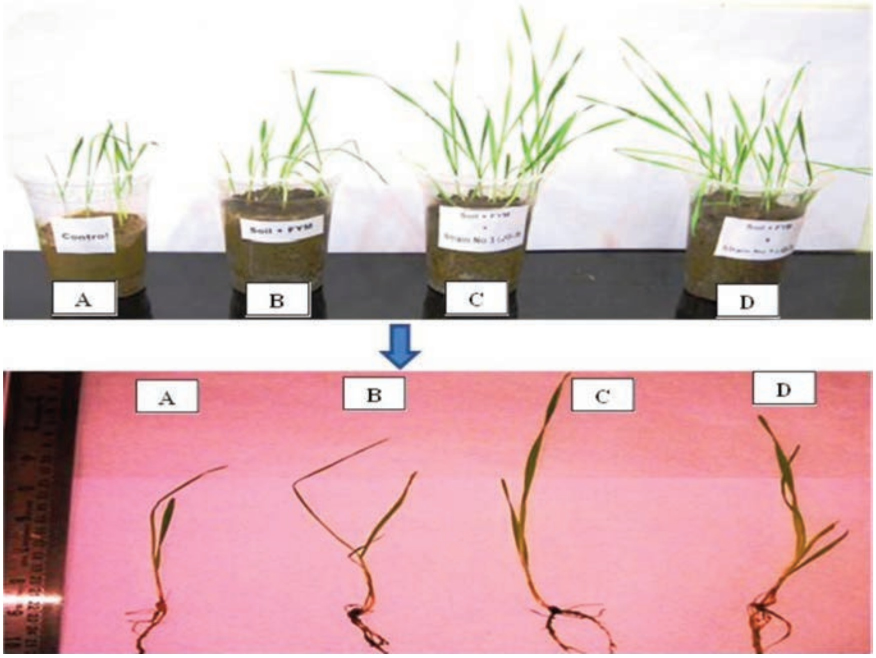

Figure 5. Comparative study of two different isolates on wheat plant growth promotion under secondary soil salinity.

Table 3. Carbohydrate utilization of rhizo-bacterial strains.

Carbohydrate utilization	JG-1	JG-2	JG-3	JG-4	JG-5	JG-6	JG-7
Lactose	+	+	+	-	+	-	+
Xylose	+	+	-	-	-	-	+
Maltose	+	-	-	+	-	+	+
Fructose	+	-	+	+	+	+	+
Dextrose	+	-	-	+	-	-	-
Galactose	-	+	-	-	-	+	-
Raffinose	-	-	-	-	-	-	-
Trehalose	-	+	+	+	+	-	-
Melibiose	-	+	+	-	-	+	-
Sucrose	-	-	+	+	+	-	+
L-Arabinose	-	-	-	-	-	-	-
Mannose	-	+	+	-	+	-	-

Table 4. Plant growth promoting activities of isolates rhizo-bacterial strains.

PGP traits of isolated strains	JG-1	JG-2	JG-3	JG-4	JG-5	JG-6	JG-7
IAA production	+	++	++	+	+	-	+++
Nitrogen fixation	-	-	+++	++	++	-	++
Siderophore production	-	-	++	-	-	-	-
Phosphate solubilization	-	+	-	-	-	-	-
Ammonia production	+	+	+	+	+	+	++
HCN production	-	+	+	-	-	-	+++

(+++ = excellent positive, ++ = moderate positive, + = slightly positive, - = negative).

ent study revealed that the variations in plant growth parameters between non-inoculated and inoculated plants with JG3 and JG7 were statistically significant (Figure 6). Therefore, it may be concluded the the application of ST-PGPR is innovative and eco-friendly approach for reclamation of degraded agro- ecosystems. Decline demand of agrochemical in crop fields protects soil health and ease detrimental effects on human health.[26]

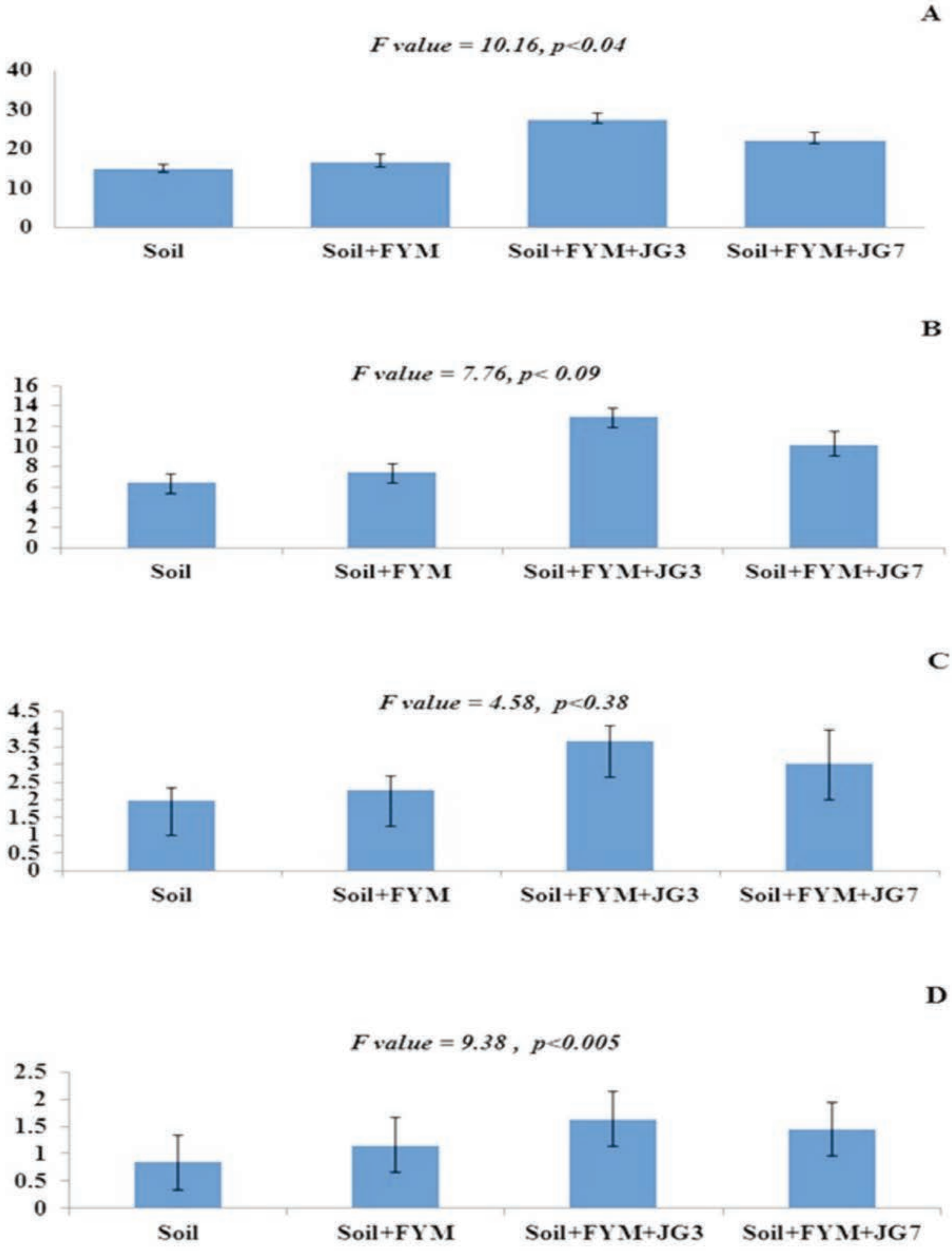

Figure 6. Inoculation effect on plant morphology (A) Plant height (B) Root length (C) Fresh weight (D) Dry weight.

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Soil-Plant-Microbe Interactions in Stressed Agriculture Management: A Review

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ABSTRACT

The expected rise in temperature and decreased precipitation owing to climate change and unabated anthropogenic activities add complexity and uncertainty to agro-industry. The impact of soil nutrient imbalance, mismanaged use of chemicals, high temperature, flood or drought, soil salinity, and heavy metal pollutions, with regard to food security, is increasingly being explored worldwide. This review describes the role of soil-plant-microbe interactions along with organic manure in solving stressed agriculture problems. Beneficial microbes associated with plants are known to stimulate plant growth and enhance plant resistance to biotic (diseases) and abiotic (salinity, drought, pollutions, *etc.*) stresses. The plant growth-promoting rhizobacteria (PGPR) and mycorrhizae, a key component of soil microbiota, could play vital roles in the maintenance of plant fitness and soil health under stressed environments. The application of organic manure as a soil conditioner to stressed soils along with suitable microbial strains could further enhance the plant-microbe associations and increase the crop yield. A combination of plant, stress-tolerant microbe, and organic amendment represents the tripartite association to offer a favourable environment to the proliferation of beneficial rhizosphere microbes that in turn enhance the plant growth performance in disturbed agro-ecosystem. Agriculture land use patterns with the proper exploitation of plant-microbe associations, with compatible beneficial microbial agents, could be one of the most effective strategies in the management of the concerned agriculture lands owing to climate change resilience. However, the association of such microbes with plants for stressed agriculture management still needs to be explored in greater depth.

Key Words: beneficial microbes, fungi, microbial agents, mycorrhiza, organic manure, pathogen, plant health, plant growth-promoting rhizobacteria

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INTRODUCTION

The primary challenge in agricultural sciences is to develop technologies that not only increase crop yield, but also endow with nutritional security and sustainability of agriculture, especially under constrained environments (Gepstein and Glick, 2013; Patel *et al.*, 2015; Hamilton *et al.*, 2016). The current agricultural practices, which heavily rely on the extensive use of agrochemicals for high yield, also lead to environmental disturbances (Singh *et al.*, 2011; Paul and Lade, 2014; Singh, 2015a). Consequences of the on-going rise in human population, dramatic change in global climate, shrinking agricultural lands, rapid urbanization, and extensive use of agrochemicals have collectively affect crop production worldwide (Glick, 2014; Rashid *et al.*, 2016). Besides, climate change and erratic weather are the two most challenging issues confronting mankind today (Ahmed *et al.*, 2015).

Escalating environmental concerns and global hunger open the door for lucrative interest in environment-friendly, sustainable, and climate-smart agricultural technologies (Singh *et al.*, 2011; Rashid *et al.*, 2016). While the control of intensifying human population is a sluggish process, escalating global hunger has knocked the brains of researchers for suitable answers. Thus, increases in the agricultural productivity seem to be the only answer to insuring food security. With no enough room to expand areas of cultivation, a critical supervision of the available fertile land seems to be a good strategy to manage agricultural productivity, ensure economic growth, protect biodiversity, and meet the increasing food demands of incessant rising global population.

The role of microorganisms in improving nutrient availability to plants is an important strategy and related to climate-smart agricultural practices (Pereg and McMillan, 2015; Hamilton *et al.*, 2016). Beneficial

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interactions have been reported among plants and microorganisms in the environment and the derived ecosystem functions (Cosme and Wurst, 2013; Nadeem *et al.*, 2014; Rashid *et al.*, 2016; Singh *et al.*, 2016a, b, c). Root exudates are responsible for rich microbial diversity around the root zone (Singh and Strong, 2016). They provide nutrition to the microbes which in turn endorse plant growth using different growth-promoting attributes (Patel *et al.*, 2015). The plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi are well known for their unique plant growth-promoting capability under stressed environments (Singh *et al.*, 2011; Meier *et al.*, 2012; Singh, 2013; Nadeem *et al.*, 2014; Kumar *et al.*, 2015; Bach *et al.*, 2016). The PGPR facilitate the plant growth through diverse mechanisms, which include acquisition of resources (Bhattacharyya and Jha, 2012), enhancement of transformation and acquisition of nitrogen (N) (Bell *et al.*, 2015), mineralization of organic phosphorus (P) (Bhattacharyya and Jha, 2012), production of phytohormones (Kurepin *et al.*, 2015), synergism with other bacteria-plant interactions (Rashid *et al.*, 2016), and mitigation of plant stresses (Singh, 2015a; Vimal *et al.*, 2016). They can also protect plants through the control of soil- and seed-borne phytopathogens (Bach *et al.*, 2016) and induction of systemic resistance (Jain *et al.*, 2013), and the production of volatile compounds (Bhattacharyya *et al.*, 2015) that can inhibit the growth of plant deleterious microbes. Most vascular plants on the earth form symbiotic associations with mycorrhizal fungi (Hashem *et al.*, 2015). Mycorrhizal associations benefit the agroecosystems using growth-promoting attributes (Smith and Read, 2008) such as improved dinitrogen (N_2) fixation by collaborating with rhizobia (Krapp, 2015), synthesis of bioactive compounds (Goicoechea *et al.*, 1997), enhanced photosynthetic rates (Ruíz-Sanchez *et al.*, 2011; Hashem *et al.*, 2015), enhanced phosphatase activity (Liu *et al.*, 2015), osmotic adjustments under stress thus enhancing productivity of marginalized soils (Jain *et al.*, 2013; Xun *et al.*, 2015), metal detoxification (Amir *et al.*, 2013; Zong *et al.*, 2015), and increased resistance against biotic (Yuan *et al.*, 2016) and abiotic stresses (Fabbro and Prati, 2014; Hashem *et al.*, 2015). The PGPR-mycorrhiza interactions represent the intimate interface with the host plants and promote plant health through suppressing the plant pathogens under stressed environments (Kohler *et al.*, 2010; Barnawal *et al.*, 2014; Sundram *et al.*, 2015). The PGPR and mycorrhiza display multiple mechanisms and roles in enhancing plant growth and health, combating phytopathogens and helping in coping under abiotic stressed conditions. Simultaneous working of diverse mechanisms by plant growth-promoting microbes (including both bacteria and fungi) under natural conditions can not be considered as their specific role and thus may dilute the concept of classifying them as direct or indirect mechanisms (Arora *et al.*, 2013). The PGPR and mycorrhizal associations can enhance the plant growth and simultaneously protect it from diseases including those from phytopathogens and deficiencies even under stress conditions/soils.

Organic amendments are important in soil nutrient management including the macro- and micronutrient status of the soils (Barnawal *et al.*, 2014). Plant-microbe-manure tripartite interactions may have vital roles in sustainable stressed agricultural management because such associations play an imperative role in improving performance of crop plants. The PGPR and mycorrhizal inoculations have been reported to be helpful in reducing the use of agrochemicals and in restoring soil health (Rashid *et al.*, 2016). Microbes improve the efficiency of applied fertilizers and manure and also the crop yields (Singh *et al.*, 2011; Rashid *et al.*, 2016). Organic amendments not only improve the soil physico-chemical status, but also increase the possibility of viability and survival of novel bio-inoculants for the reclamation of stressed agriculture and eco-restoration (Rashid *et al.*, 2016). Land use changes and the associated loss of beneficial microbial diversity are the major reasons for deterioration of soil fertility and agricultural productivity (Singh *et al.*, 2010; Singh and Singh, 2012; Singh, 2014). Therefore, the areas related to plant-microbe associations to combat problems of stressed agriculture need to be investigated in greater depth. There is also a need to explore the diversity of stress-tolerant microbes in relation to host plant species, their habitat, and the geographical locations for management of stressed agriculture. This review comprehensively summarizes the possible plant-microbe interactions along with amendment of soils with organic manure for improving plant fitness and combating stressed agricultural problems. The possible roles of beneficial microbes in combination with amendment of soils with organic manure in management of stressed agriculture are shown in Fig. 1.

ENVIRONMENTAL STRESSES AND PLANT HEALTH

Abiotic and biotic stresses directly affect agricultural productivity. High salinity and temperature suppress plant growth and reduce crop yield. For survival, plants have to adapt and acclimatize to their surrounding environment, but the metabolic activities get dis-

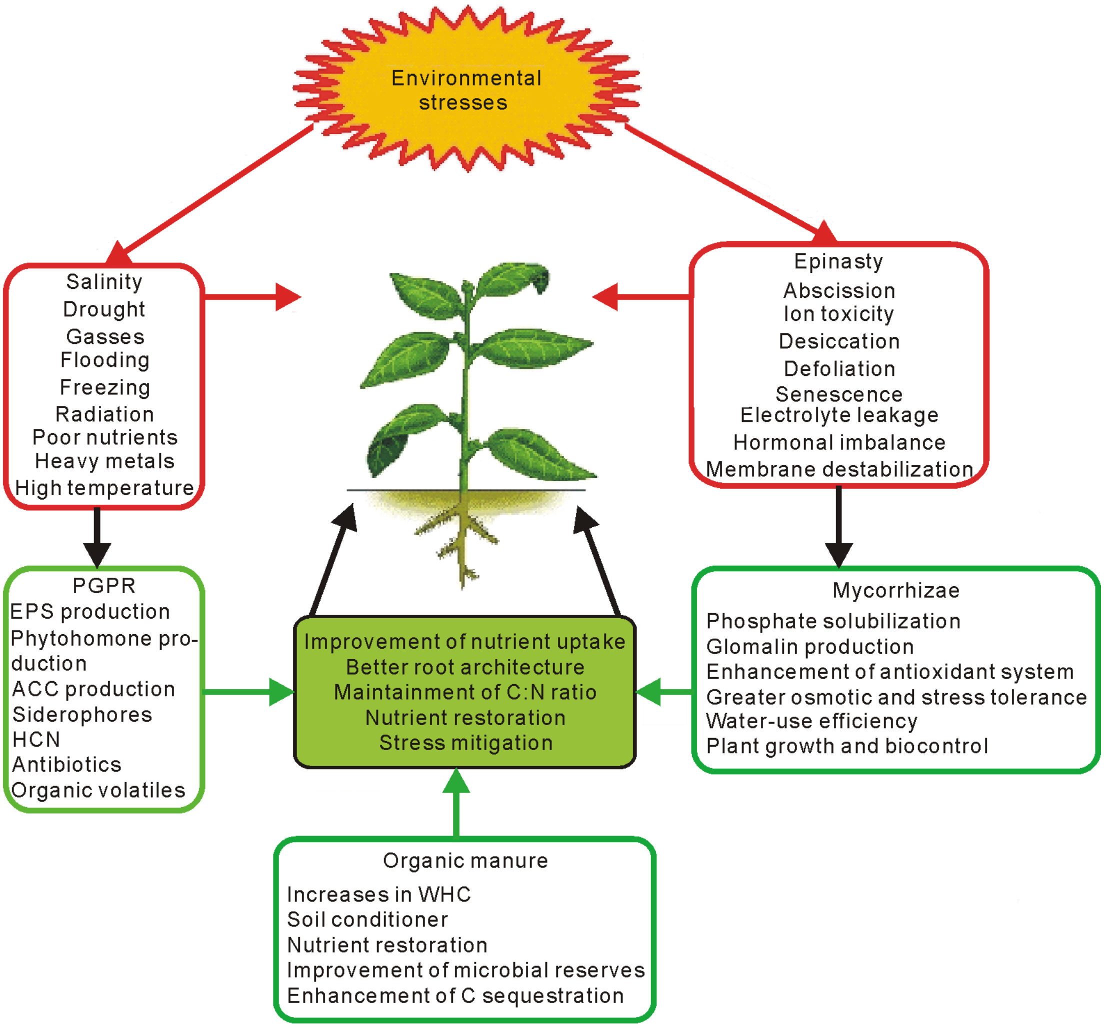

Fig. 1 Possible roles of beneficial microbial agents along with organic manure in stressed agriculture and soil management. PGPR = plant growth-promoting rhizobacteria; EPS = exopolymeric substance; ACC = 1-aminocyclopropane-1-carboxylate; HCN = hydrogen cyanide; WHC = water-holding capacity.

turbed when the intensities cross the limit (Paul and Lade, 2014). The stresses impacting plant growth include hormonal and nutritional imbalance, physiological disorders such as epinasty, abscission, senescence, and susceptibility to diseases (Glick, 2014; Paul and Lade, 2014). Under stressed environments, plants produce elevated levels of ethylene (C_2H_4), which at higher concentrations imparts drastic effects on plant health, including defoliation and other unbalanced cellular processes (Barnawal *et al.*, 2014; Glick, 2014).

Salinity, one of the most serious factors limiting agricultural productivity, adversely affects germination, plant vigour, and crop yield (Paul and Lade, 2014), which becomes more prevalent especially in the arid and semi-arid regions of the world. High salt concentrations cause an imbalance of cellular ions, resulting in ion toxicity, osmotic stress, and the production of reactive oxygen species (ROS) (Grover *et al.*, 2011). In addition, under salinity stress, ion toxicity occurs, which is particularly due to excess accumulation of Na^+ and Cl^- that causes adverse effects on plant growth and development (Paul and Lade, 2014). Excess Na^+ and more importantly Cl^- affect plant enzymes and cause cell swelling, reducing energy production along with other physiological changes (Nunkaew *et al.*, 2015). The uptake and accumulation of Cl^- disrupt photosynthetic functions through inhibition of nitrate reductase (NR) activity (Nadeem *et al.*, 2014). Once the capacity of cells to store the salts is exhausted, their build-up in the intracellular space leads to cell dehydration and death (Kang *et al.*, 2014). During the onset of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis, energy, and lipid metabolism are affected (Paul and Lade, 2014; Hashem *et al.*, 2015). Photosynthetic capacity is considerably reduced due to the osmotic stress and the partial closure of stomata (Hashem *et al.*, 2015). Plants experience membrane destabilization and endure nutrient imbalance (Nadeem *et al.*, 2014). Furthermore, plant responses to osmotic stress are reflected in terms of decreased cell growth and development, reduced leaf area and chlorophyll content, and accelerated defoliation and senescence (Kang *et al.*, 2014).

Similarly, drought stress changes chlorophyll contents and damages the photosynthetic apparatus in plants (Ortiz *et al.*, 2015). Root growth is severely affected by limited supply of water. Similarly, the stresses such as salinity, heavy metals, nutrient deficiency/excess, and pathogen attack also cause negative impact on plant growth and development in a number of ways that may include hormonal imbalance and susceptibility to diseases and metal toxicity (Miransari, 2011; Nadeem *et al.*, 2014).

In nature, plants are sensitive to changes in temperature, and respond to seasonal variations and more so to seasonal diurnal changes. The heat stress in terms of so-called global warming is a serious threat to world agriculture (Liu *et al.*, 2016). A fluctuation in temperature may lead to hormonal imbalances in plants and also to their growth (Grover *et al.*, 2011). Heat-stressed crops may suffer from dehydration, depressed growth, and decline in harvest yield (Liu *et al.*, 2016). As evident from the observations mentioned above, abiotic and biotic stresses are detrimental to the plant growth. These stresses affect plant growth and development through adversely affecting the morphological, physiological, and biochemical processes and, ultimately, the yield.

PLANT-MICROBE INTERACTIONS IN MANAGING STRESSED AGRICULTURE

Environmental stresses and their variability are the major factors that influence the agricultural outputs by disturbing rhizosphere functioning (Singh, 2015a). A healthy plant rhizosphere not only is helpful in nutrient and water facilitation to plants, but also provides sustained benefits to the microbial diversity, which collectively facilitates the plant health. Plant genotypes are mainly responsible for the composition of root exudates that account for the microbial recruitment in the rhizosphere region (Patel *et al.*, 2015). The composition of the beneficial microbial diversity around the root zones imparting improved plant growth-promoting attributes includes rhizobacteria, mycorrhizal fungi, and other microbes (Nadeem *et al.*, 2014; Hamilton *et al.*, 2016). These beneficial microorganisms interact with plant roots symbiotically and support plant health *via* myriad of mechanisms, such as control of pathogens, production of secondary metabolites, and increases in resistance against stresses (Glick, 2014; Rashid *et al.*, 2016). Beneficial fungal networks around the root zones protect the plants against various pathogens, and thus act as the potential bio-control agents (Fabbro and Prati, 2014). The fungal networks enhance P acquisition and water availability under the condition of severe water scarcity (Barnawal *et al.*, 2014). Thus, plant-microbe symbiosis could be the viable association which could undoubtedly enhance the acquisition of essential nutrients and moisture by plants, uplift usable forms of N, release growth-promoting hormones, enzymes, metabolites, and organic volatiles, and nurture plant health.

Plant-N_2 fixer symbiosis in soil conditioning

Nitrogen is one of the key nutrients necessarily required for the improvement of growth and nutritional contents of plants (Krapp, 2015). The role of N in chlorophyll biosynthesis and photosynthesis in plants is well known (Zimmer *et al.*, 2016). Actively participating in many physiological and biochemical processes in plants (Krapp, 2015), N is reported to be the major nutrient required in sufficient amounts to sustain crop yield and quality (Sainju, 2013). However, the excessive use of agrochemicals in modern agricultural practices drastically increases the anthropogenic N loading as the major amount of the N applied to crop plants is not consumed (Yang *et al.*, 2014). Thus, excessive use of nitrogenous fertilizers not only is economically expensive, but also leads to a cascade of large-scale environmental impacts, including threatening of the ecosystem sustainability around the world through terrestrial and aquatic eutrophication and acidification and creating of large hypoxic zones (Galloway *et al.*, 2008; Yang *et al.*, 2014). Microbes are also reported to play important roles in soil N stress management. Biological N_2 fixation (BNF) mediated by microbes contributes 180×10^6 t of fixed N per year globally, out of which 80% is contributed by symbiotic associations and the rest comes from free-living or associative systems (Graham, 1988). Fixation of N_2 is a highly energy-extensive process, requiring at least 16 mol of adenosine triphosphate (ATP) for each mole of elemental N_2 reduced, and it would be advantageous if the bacterial carbon (C) resources are directed toward oxidative phosphorylation which favours ATP synthesis, rather than glycogen synthesis, as the latter results in storage of energy as glycogen (Glick, 2012).

Bell *et al.* (2015) ascertained that organic constituent inputs in rhizosphere through root secretions alter microbial diversity that determines the plant N uptake. In agricultural settings, perhaps 80% of the biologically fixed N comes from *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium* of the family Rhizobiaceae in association with the leguminous plants. *Rhizobium* and *Bradyrhizobium* establish symbiotic associations with roots in leguminous plants such as soybean, pea, peanut, and alfalfa, convert N_2 into ammonia, and make it available to the plants as a source of N (Badawi *et al.*, 2011). The PGPR which reside in the inner plant part are termed as intracellular PGPR (i-PGPR). These PGPR form special nodular structure to enhance the rate of N_2 fixation (Gray and Smith, 2005). Endophytes, which include a wide range of soil bacterial genera such as *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium*, and *Rhizobium* of the family Rhizobiaceae, generally invade the root systems of crop plants to form nodules (Zimmer *et al.*, 2016) and stimulate plant growth either directly or indirectly. The PGPR found on the rhizoplane or within the apoplast of the root cortex are termed as extracellular PGPR (e-PGPR). The e-PGPR include *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas*, and *Serratia* (Gray and Smith, 2005). The i-PGPR include the endophytes and *Frankia* species, both of which can symbiotically fix N_2 in association with the higher plants (Badawi *et al.*, 2011).

It has been reported that most of the plants form mycorrhizal symbiosis, mainly dicots and monocots (Smith and Read, 2008). The main mycorrhizal groups explored to form symbiotic associations with plants are as follows: arbuscular mycorrhizae, ectomycorrhizae, ectendomycorrhizae, arbutoid mycorrhizae, ericoid mycorrhizae, monotropoid mycorrhizae, and orchid mycorrhizae (Smith and Read, 2008). N_2 fixation, the key input of N for plant productivity, is the first step in cycling of N to the biosphere from the atmosphere (Nadeem *et al.*, 2014). Fungi show enhanced N status when inoculated with rhizobia (Barrett *et al.*, 2015), PGPR (Armada *et al.*, 2015) or both (Barnawal *et al.*, 2014). Symbiotic N_2 fixation is the well-known process exclusively driven by bacteria, the only organisms possessing the key enzyme nitrogenase, which specifically reduces N_2 to ammonia in the symbiotic root nodules (Zimmer *et al.*, 2016). The *nif* genes (nitrogenase genes) are N_2 fixation genes and are present in both symbiotic and free-living systems (Kim and Rees, 1994). These genes include structural genes, genes involved in the activation of iron proteins, iron-molybdenum cofactor biosynthesis, and electron donation, and regulatory genes required for the synthesis and function of enzymes. In diazotrophs, the *nif* genes are typically found in a cluster of around 20–24 kb with seven operons encoding 20 different proteins (Glick, 2012). The activation of *nif* genes in the symbiotic *Rhizobium* is dependent on low concentrations of oxygen, which in turn is regulated by another set of N_2 fixation genes, *fix* genes, which are common to both symbiotic and free-living N_2-fixing systems (Kim and Rees, 1994). The organic amendments traditionally used by the farmers to enhance crop productivity are cheaper and easily available, but not so effective like those of agrochemicals in crop promotion. Organic farming is the hope for environmental protection and ecosystem restoration; however, it has been argued that organic or agro-ecology farming systems are good for the environment, but these are often associated with lower yields when compared to the conventional farming systems (Seufert *et al.*, 2012). It is therefore expected that enrichment of organic amendments with various effective and naturally occurring microbial strains will be an effective, safe, viable, clean, and green technology to improve soil health and sustainability.

Plant-microbe associations in nutrient uptake

Mycorrhizal fungi represent a significant portion of the soil flora and appreciably influence plant growth and development in nutrient-stressed soils. The symbiotic associations of fungi with plant roots increase the root surface area and thereby enable the plants to absorb water and nutrients more efficiently from the large soil volume (Barzana *et al.*, 2012; Kaiser *et al.*, 2015). The arbuscular mycorrhizal (AM) fungi (AMF) and ectomycorrhizal (EcM) fungi (EcMF) form symbiotic associations with most terrestrial plants (Porcel *et al.*, 2012). Both AMF and EcMF support extensive extraradical hyphal networks which act as the conduit for nutrient exchange between plant roots and the soil environment (Buscot, 2013). About 80% of the plant species form associations with glomeromycotan fungi, which penetrate into their root cortex and grow intercellularly before forming the highly branched haustoria-like structures (arbuscules) (Buscot, 2015). This trait leads to such associations being called as AMF (Nadeem *et al.*, 2014). The AMF are probably the most abundant fungi commonly present in agricultural soils, and arbuscules are the main sites for the exchange of P, N, and other minerals mobilized by the thin fungal hyphae in soils (Rashid *et al.*, 2016). The efficient exchange of nutrients (*e.g.*, sucrose to the fungus and N/P to the plant) is mediated *via* intracellular arbuscules in AMF (Rashid *et al.*, 2016). The ability of fungi to produce fine hyphae (with more favourable surface area to volume ratio for nutrient uptake) and to secrete enzymes/organic acids to mobilize the nutrients is the basis of mutualism (Owen *et al.*, 2015). Their large surface area makes the fine hyphae produced more effective than plant root hairs in mineral uptake from the soils (Cavagnaro *et al.*, 2015). The AMF are ecologically important soil microbes; they form obligate symbiosis with roots of most terrestrial plants (Smith and Read, 2008). In symbiosis, host plants provide fixed C to the AMF and in return derive several benefits such as greater nutrient uptake, drought and salt tolerance, metal stress alleviation, and resistance to pathogen and biotic stresses (Smith and Read, 2008). Thus, mycorrhizal roots can explore more soil volume because their extramatrical hyphae facilitate absorption and translocation of more nutrients than those of nonmycorrhizal plants (Ortiz *et al.*, 2015). The AMF can also redistribute resources (C, N, and P) between plants, alter their competitive interactions (Rashid *et al.*, 2016), and thus drive the plant population dynamics and community processes (Smith *et al.*, 2011). An enormous amount of P taken up by the plants in P-poor soils is supplied by the AMF (Marschner and Dell, 1994; Cavagnaro *et al.*, 2015). In addition to their important role in P acquisition, the AMF also provide other macro- and micronutrients such as N, potassium (K), magnesium (Mg), copper (Cu), and zinc (Zn), particularly to soils where these are present in the less soluble forms (Smith and Read, 2008). It has been reported that up to 60% of Cu, 25% of N, 25% of Zn, and 10% of K in a plant can be delivered by the external AMF hyphae (Marschner and Dell, 1994; Hodge and Storer, 2015). Mycorrhizae also enhance soluble sugars and electrolyte concentrations in the host plants. For example, improved osmoregulation capacity in AMF-inoculated maize was related to higher soluble sugar and electrolyte concentrations (Feng *et al.*, 2002; Gosling, 2006). The AMF such as *Glomus intraradices, Glomus mosseae*, and *Glomus caledonium* improve crop performance under drought conditions (Ortiz *et al.*, 2015), and help in alleviating salt- (Hashem *et al.*, 2015) and heavy metal-induced oxidative stresses (Zong *et al.*, 2015; Wu *et al.*, 2016). The possible beneficial plant-microbe and microbe-microbe interactions in sustainable stressed agriculture and soil management have been proposed as in Fig. 2.

The EcMF consist of both fine roots and fungal tissues and create a unique and metabolically active habitat within the soil ecosystems (Kernaghan and Patriquin, 2015). The EcM mycelial systems are also known to be the essential component involved in nutrient capture and C turnover (Smith and Read, 2008). The EcMF develop variously structured hyphal sheaths (mantle) around the roots and differently organized mycelia that emanate from the mantle (extramatrical mycelium). The extramatrical mycelia grow either as the simple hyphae from the mantle scattered into the soil or can get united to form undifferentiated rhizomorphs with the smaller ones reaching to highly organized root-like organs (vessel-like hyphae) for efficient water and nutrient transport from the distance of decimetres (Kernaghan and Patriquin, 2015). The host

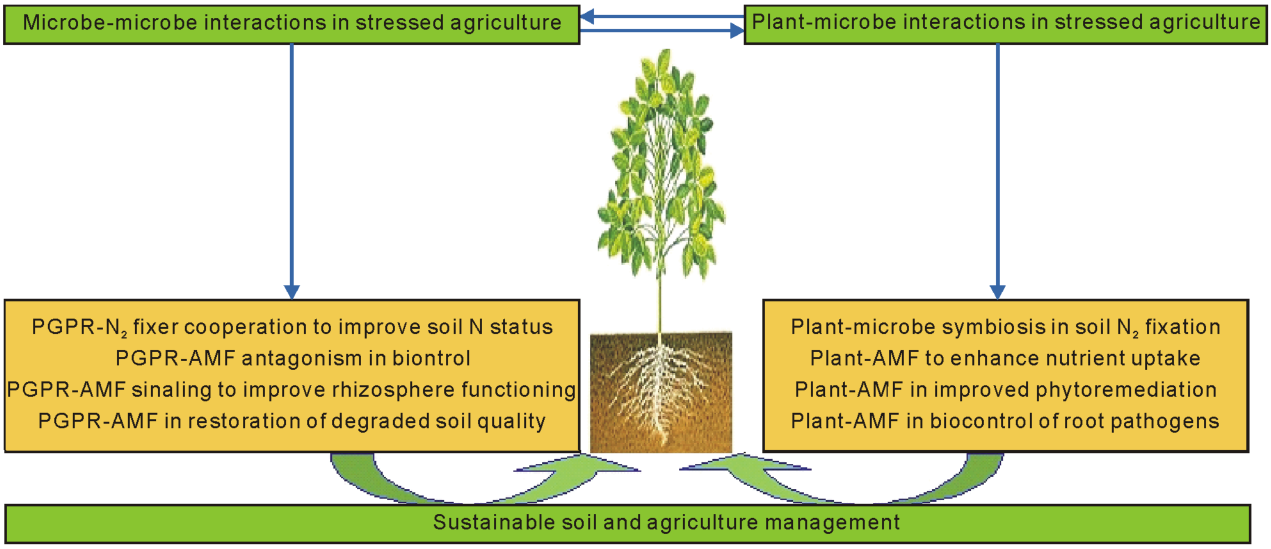

Fig. 2 Possible beneficial plant-microbe and microbe-microbe interactions in sustainable stressed agriculture and soil management. PGPR = plant growth-promoting rhizobacteria; AMF = arbuscular mycorrhizal fungi.

plant is privileged to get more nutrients and protection without the expenditure of extra energy because of the AMF hyphae that have access to nutrient-rich zones. Therefore, the use of mycorrhizae in nutrient-poor and stressed soils, *i.e.*, arid and semi-arid conditions, mine spoiled degraded soils, and fly ash dump sites, has become an attractive option since the symbiotic associations with these fungi enable the plants to increase their nutrient uptake and water status and reduce the need for external inputs of agrochemicals for the improved plant health under stressed environments.

Plant-microbe associations in phytoremediation

In the recent decades, increasing industrialization and urbanization and inadequate disposal of wastes have resulted in an increase in the heavy metal concentrations in agriculture soils (Singh and Singh, 2012; Wu *et al.*, 2016). Heavy metal pollution of the soils and other ecosystems gets gradually amplified because of the intense anthropogenic activities and poses serious health problems and disturbances in ecosystem functioning and the food chain owing to biomagnifications (McMichael *et al.*, 2015). Mycorrhizal fungi are the important components of ecosystem because they significantly increase the plant efficiency to accumulate nutrients including heavy metals from the soil (Liu *et al.*, 2015). Bioremediation (phytoremediation) in association with mycorrhizal fungi is an emerging tool for removal of such soil pollutants and to ensure sustainable agriculture. Mycorrhizae-mediated uptake of metal pollutants (phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization) has been used efficiently for the decontamination of soils (Miransari, 2011). The mycorrhizal fungi interact with plants in metal-contaminated soils and enable them to survive in such soils (Wu *et al.*, 2016). The fungal cell walls contain free amino acids, hydroxyls, carboxyls, and other functional groups that act as binding sites for the adsorption of certain trace elements. The AMF are considered the most crucial endomycorrhizal fungi for phytoremediation (Wu *et al.*, 2016). Metal-accumulating plants in association with AMF showed greater ability for metal absorption and translocation (Meier *et al.*, 2012). The AMF enhance the availability of P and other nutrients to plants in contaminated environments (Rashid *et al.*, 2016). The host plant *Solanum nigrum* on Cd-contaminated soils can accumulate significantly higher levels of cadmium (Cd) when inoculated with an AMF *Glomus versiforme* (Liu *et al.*, 2015). Meier *et al.* (2011) have also suggested the relevant role of a glomeromycotan fungus, *Glomus claroideum*, isolated from Cu-polluted environments in the alleviation of Cu toxicity, and indicated their use in remediation programs of Cu-polluted soils. Ectomycorrhizae predominantly host dicots, mainly the trees of families *Pinaceae, Fagaceae, Dipterocarpaceae*, and *Caesalpinoidaceae*, distributed in tropical, subtropical, temperate, and boreal forests (Smith and Read, 2008). Ectomycorrhizal hyphal networks form Hartig net, the interface for exchange of water, nutrients, and other compounds between fungi and plants (Henke *et al.*, 2015). Following the uptake by ectomycorrhizal fungal cells, nutritional heavy metal ions get transported to the Hartig net, translocated out of the fungal cells into root cells, and further transported to the other parts of the host plant with the help of plant metal transporters (Luo *et al.*, 2014). The ectomycorrhizal mechanisms for detoxification and sequestration of heavy metals have been reported in the associated plants (Henke *et al.*, 2015). Plant root exudates play an important role in metal sequestrations (Meier *et al.*, 2012). The enhanced tolerance of *Pinus densiflora* seedlings to Cu-stressed mine soils was primarily due to the increased nutrient uptake and the inhibition of the translocation of heavy metals by the EcMF *Pisolithus* sp. (Zong *et al.*, 2015). Since the application of organic manure in the contaminated soils helps in solubilizing heavy metals, the enrichment of soil with manure and efficient microbes may offer a viable tool for heavy metal removal from the stressed agriculture soils (Rashid *et al.*, 2016). Thus, bioremediation of heavy metals using plant-microbe associations along with organic manure may be a safe and effective tool in the management of stressed agriculture. Some examples of plant-microbe associations in phytoremediation studies are illustrated in Table I.

Plant-microbe interactions in managing plant pathogens

Microbe-mediated control of plant pathogens is currently accepted as a key practice for the management of crop destructive diseases (Romeralo *et al.*, 2015). Plant-AMF associations reduce the damage caused by soil-borne plant pathogens (Table II). Nutrient uptake of plants *via* their AM fungal symbionts (*e.g.*, *Glomus intraradices* and *Glomus mosseae*) improved their tolerance against pathogenic infections (Bach *et al.*, 2016). Higher fungal biomass around the root zones of plants also enhances competitive interactions with the pathogenic fungi. These interactions have been suggested as the mechanisms through which AMF reduce the abundance of pathogenic fungi in the rhizosphere. Chagnon and Bradley (2015) suggested that plant hor-

TABLE I

Plant-microbe associations in phytoremediation studies

Study	Plant(s)	Microbe(s)	Heavy metal(s)	Combined effect
Zong *et al.* (2015)	*Pinus densiflora*, *Quercus variabilis*	*Pisolithus* sp., *Cenococcum geophilum*, *Laccaria laccata*	Cu	Enhanced heavy metal accumulation in roots, reduced heavy metal accumulation in shoots
Liu *et al.* (2015)	*Solanum nigrum*	*Glomus versiforme*	Cd	Enhanced phosphatase activity, higher heavy metal uptake
Lermen *et al.* (2015)	*Cymbopogon citratus*	*Rhizophagus clarus*	Pb	Enhanced productivity of essential oils from plants
Amir *et al.* (2013)	*Alphitonia neocaledonica*, *Cloezia artensis*	*Glomus etunicatum*	Ni	Enhanced P uptake and biomass production, reduce heavy metal concentration in roots and shoots
Meier *et al.* (2011)	*Oenothera picensis*	*Glomus claroideum*	Cu	Decreased antioxidant status at different heavy metal levels, enhanced mycorrhizal colonization
Gonzalez-Chavez *et al.* (2002)	*Sorghum vulgare*	*Glomus caledonium*, *Glomus claroideum*, *Glomus mosseae*	Cu	Increased heavy metal absorption rate
Punaminiya *et al.* (2010)	*Chrysopogon zizanioides*	*Glomus mosseae*	Pb	Increased plant biomass, heavy metal uptake by plant roots and its translocation to plant shoots, elevated chlorophyll and thiol contents
Pongrac *et al.* (2009)	*Thlaspi praecox*	*Phialophora verrucosa*, *Rhizoctonia* sp., *Penicillium brevi compactum*, *Rhodotorula aurantiaca*	Cd, Pb, Zn	Enhanced heavy metal uptake by plant roots due to greater densities of mycorrhizal vesicles and microsclerotia
Wang *et al.* (2007)	*Zea mays*	*Glomus caledonium*, *Gigaspora margarita*, *Gigaspora decipiens*, *Scutellospora gilmorei*	Cu, Zn, Pb, Cd	Increased P uptake with higher mycorrhizal covering, promoted extraction efficiencies of heavy metals

monal interactions with the pathogens play an important role in the strong association between AMF and plants. Inoculations with an AMF (*Glomus intraradices* or *Glomus mosseae*) modify the bacterial community of tomato rhizosphere against the soilborne pathogen *Phytophthora nicotianae* (Lioussanne *et al.*, 2009). Thus, it appears that the supportive effects of AMF on rhizosphere bacteria are not mediated by the compounds in root exudates of mycorrhizal plants, but rather by the physical or chemical factors associated with the mycelia, volatiles, and/or root surface-bound substrates.

The AMF have also exhibited potential biocontrol activities against various nematodes (Vos *et al.*, 2012). The AMF *Glomus mosseae* exhibited potent systemic resistance against two nematodes (*Meloidogyne incognita* and *Pratylenchus penetrans*) in association with *Lycopersicum esculantum* (Vos *et al.*, 2012). The mycorrhiza-induced disease resistance against both the nematode species was ascertained as the nematode population was significantly lowered in mycorrhizal roots, with an overall reduction of 45% and 87% in the cases of *Meloidogyne incognita* and *Pratylenchus penetrans*, respectively. Endophytic fungal filtrates are effective against *Gremmeniella abietina* infections in Aleppo pine seedlings (Romeralo *et al.*, 2015). Rabiey *et al.* (2015) studied the suppression of fungal pathogen *Fusarium culmorum* or *Fusarium graminearum* causing crown rot disease in *Triticum aestivum* through the root endophytic fungus *Piriformospora indica.* The EcMF also suppress various plant pathogens (Ismail *et al.*, 2011). Pine trees (*Suillus luteus*) in association with EcMF antagonize the effect of fungal pathogens (*Heterobasidion irregulare* and *Heterobasidion annosum*) (Sillo *et al.*, 2015). The application of *Trichoderma harzianum*-amended bioorganic fertilizer or *Glomus mosseae* alone significantly reduced the abundance of *Ralstonia solanacearum* in the rhizosphere soils; however, the integrated treatment (*Trichoderma harzianum* with *Glomus mosseae*) had the strongest inhibitory effect, indicating that higher mycorrhizal colonization and systemic resistance against pathogens can improve the plant biomass (Yuan *et al.*, 2016). Some significant reports related to roles of plant-fungi interactions in controlling plant pathogens are listed in Table II.

MICROBE-MICROBE INTERACTIONS IN STRESSED AGRICULTURE MANAGEMENT

Microbe-microbe interactions affect plant health either mutualistically or antagonistically. Microbes gro-

TABLE II

Plant-microbe interactions in controlling plant pathogens in some significant studies

Study	Host plant(s)	Microbe(s)	Pathogen(s)	Combined effect
Yuan *et al.* (2016)	*Nicotiana tabacum*	*Trichoderma harzianum, Glomus mosseae*	*Ralstonia solanacearum*	Reduced disease incidence, enhanced systemic resistance, improved plant height, and shoot and root dry weights
Fabbro and Prati (2014)	*Senecio vernalis*, *Senecio inaequidens*, *Inula conyza*, *Conyza canadensis*, *Solidago virgaurea*, *Solidago gigantean*	*Glomus intraradices*, *Glomus mosseae*, *Glomus geosporum, Glomus claroideum*, *Glomus etunicatum*	*Pythium ultimum*	Inhibited pathogen, improved plant health
Sennoi *et al.* (2013)	*Helianthus tuberosus*	*Trichoderma harzianum, Glomus clarum*	*Sclerotium rolfsii*	Reduced disease incidence
Vos *et al.* (2012)	*Lycopersicon esculentum*	*Glomus mosseae*	*Meloidogyne incognita*, *Pratylenchus penetrans*	Significantly reduced disease development
Lioussanne *et al.* (2009)	*Lycopersicon esculentum*	*Glomus mosseae*, *Glomus intraradices*	*Phytophthora nicotianae*	Plants showing resistance to pathogen invasions
Al-Askar and Rashad (2010)	*Phaseolus vulgaris*	*Glomus intraradices*, *Glomus mosseae*, *Glomus clarum*, *Gigaspora gigantea*	*Fusarium solani*	Significantly enhanced phenolic contents and defensive enzyme activities
Khaosaad *et al.* (2007)	*Hordeum vulgare*	*Glomus mosseae*	*Gaeumannomyces graminis*	Inhibited pathogens with dense mycorrhizal colonization
Jaiti *et al.* (2007)	*Phoenix dactylifera*	*Glomus monosporus*, *Glomus deserticola*, *Glomus clarum*	*Fusarium oxysporum*	Improved plant health status, shoot height, number of leaves, and activities of defence-related enzymes

wing in nutritionally rich environments execute valuable interactions to support plant health. The PGPR and mycorrhizal fungi get diversified in plant rhizosphere. The PGPR mutualistically support hyphal growth and are termed as mycorrhizal helper bacteria (MHB) (Garbaye, 1994). Hyphal networks inhibit soil pathogens and facilitate water and nutrients to plants (Sillo *et al.*, 2015).

PGPR-fungi interactions in stressed agriculture

Microbial interactions are largely determined by various abiotic (pH, temperature, organic matter content, precipitation/moisture, exchangeable cation content, structure, recalcitrant humus, bulk density, texture, C/N ratio, resource heterogeneity, *etc.*) and biotic (competition, antagonism, predation, *etc.*) factors (Nadeem *et al.*, 2014; Hamilton *et al.*, 2016). These stresses may drastically influence the beneficial microbial activities in soils and can be a great cause for the reduction in crop yield by up to 50%–82%, depending on the type of crops (Kang *et al.*, 2014). Expected climate and land use changes can create unfavourable stress conditions to agriculture systems and overall microbial interaction-driven ecobiotechnological and ecosystem processes (Paul and Lade, 2014). The negative influence of such stress on microbial efficiency can be reduced by combined inoculation of beneficial microbes that may help to improve crop resistance against abiotic stress conditions. The inoculation of microbes that produce exopolymeric substances (EPS), such as *Pseudomonas mendocina* (Kohler *et al.*, 2006), in saline soils accelerates the binding of excess Na^+ to the soils and reduces the Na^+ available for plant uptake. Glycoprotein (glomalin) produced by the inoculation of AMF can act as an insoluble glue to stabilize soil aggregates (Rashid *et al.*, 2016). Thus, under salt-affected stressful soil conditions, co-inoculation of *Pseudomonas mendocina* and *Glomus mosseae* was a suitable ecobiotechnological approach for increasing soil aggregate stability (Kohler *et al.*, 2010). Under drought stress, reduced water uptake decreases N and C metabolism and ultimately changes plant physiology (Ruíz-Lozano *et al.*, 2011). Inoculation of AMF to drought-stressed soils can enhance plant antioxidant activity and consequently reduced oxidative damages are noted under such stressed environments (Hashem *et al.*, 2015; Kumar *et al.*, 2015). The co-inoculation of PGPR and AMF has proved to be more useful for enhancing water status and nutrient contents of rice plants in drought-affected soils (Ruíz-Sánchez *et al.*, 2011). In saline environments, PGPR and AMF prove to be helpful in providing nutrients to plants subjected to stress conditions (Nadeem *et al.*, 2014). This is further supported by the research of Barnawal *et al.* (2014) on inoculation of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-producing *Arthrobacter protophormiae* and *Rhizobium leguminosarum* with *Glomus mosseae* in *Pisum sativum.* Drought- and saline-stressed soils generate excess free radicals, which may damage cellular lipids, proteins, and DNA, and thus alter the normal physiology of plants, consequently leading to various diseases (Ahmed *et al.*, 2015). The study of Dhawi *et al.* (2015) on inoculation of *Glomus* spp. with *Pseudomonas* in *Zea mays* under metal-stressed soils showed that mycorrhizal inoculation alone or in combination with plant growth-promoting bacteria (PGPB) upregulates glyoxylate and dicarboxylate metabolism and some amino acids including those that feed into metabolic pathways. Inoculation of *Acinetobacter* sp. with *Glomus intraradices* enhances the phytoremediation performance of *Avena sativa* in saline-alkali soils contaminated by petroleum oil (Xun *et al.*, 2015). It may be argued that AMF colonization may significantly enhance rice growth with increased biomass, photosynthetic capacity, stomatal conductance, ascorbate content, proline content, and ultimately the vigour of rice plants. Thus, the co-inoculation of PGPR and mycorrhizal fungi is effective in enhancing plant growth and phytoremediation under stressed environments. This positive effect might be due to the combination of certain mechanisms and also the synergistic effect of these populations on one another. Some examples of PGPR-fungi interactions in well-functioning disturbed agro-ecosystems are listed in Table III.

PGPR-fungi communications in rhizosphere functioning

Beneficial plant-microbe communications in the root zone are the primary determinants of plant health and soil fertility. Belowground microbial communities have been suggested to harbour a wide variety of mechanisms to support plant health as well as productivity (Singh *et al.*, 2011; Singh, 2015b). Microbial interactions induce beneficial rhizosphere functioning through N_2 fixation (Nadeem *et al.*, 2014), nutrient solubilization (Rashid *et al.*, 2016), phytohormone production (Cosme and Wurst, 2013), photosynthesis efficiency (Hashem *et al.*, 2015), promotion of phytoreme-

TABLE III

Plant growth-promoting rhizobacteria (PGPR)-fungi interactions in mitigation of agriculture stresses

Study	Host plant	PGPR	Fungi	Stress	Combined effect on plant health
Sundram *et al.* (2015)	*Elaeis guineensis*	*Pseudomonas aeruginosa*	*Glomus intraradices*, *Glomus clarum*	Biotic	Significantly reduced epidemic rates of diseases in nursery seedlings pre-inoculated with a combination of AMF and PGPR
Xun *et al.* (2015)	*Avena sativa*	*Acinetobacter* sp.	*Glomus intraradices*	Petroleum	Enhanced antioxidant levels in plant leaves, enhanced oil removal and soil restoration process
Barnawal *et al.* (2014)	*Pisum sativum*	*Arthrobacter protophormiae*	*Glomus mosseae*	Salt	Enhanced plant health, reduced proline content and lipid peroxidation, increased pigment activity
Hernández-Montiel *et al.* (2013)	*Carica papaya*	*Pseudomonas* sp.	*Glomus intraradices*, *Glomus mosseae*, *Glomus etunicatum*, *Gigaspora albida*	Biotic	Reduced disease as well as pathogen colonization in seedlings
Zarea *et al.* (2012)	*Triticum aestivum*	*Azospirillum* sp.	*Piriformospora indica*	Salt	Improved plant fresh and dry weights, photosynthetic pigments, and proline accumulation
Ruíz-Sánchez *et al.* (2011)	*Oriza sativa*	*Azospirillum brasilense*	*Glomus intraradices*	Drought	Increased stomata conductance, photosynthesis, shoots fresh weight, and plant vigour
Kohler *et al.* (2010)	*Lactuca sativa*	*Pseudomonas mendocina*	*Glomus mosseae*	Salt	Enhanced plant biomass
Vivas *et al.* (2006)	*Trifolium repens*	*Brevibacillus brevis*	*Glomus mosseae*	Heavy metal	Reduced metal acquisition, increased shoot and root plant biomass, enhanced nodulation
Marulanda *et al.* (2006)	*Retama sphaerocarpa*	*Bacillus thuringiensis*	*Glomus intraradices*	Drought	Enhanced root development and water tolerance

diation (Wu *et al.*, 2016), and induction of defence mechanisms against abiotic (Nadeem *et al.*, 2014) and biotic stresses (Bach *et al.*, 2016) that collectively affect the crop productivity. Many rhizosphere-dwelling bacteria, collectively called as PGPR, also enhance plant growth by involving various mechanisms (Bhattacharyya and Jha, 2012). The growth-promoting ability of some bacteria being highly specific to a certain plant species, cultivar, and genotype has also been reported (Nadeem *et al.*, 2014; Rashid *et al.*, 2016). The AMF support plant nutrition by absorbing and translocating mineral nutrients beyond the depletion zones of plant rhizosphere and induce changes in the secondary metabolism for the improved yield of nutraceuticals (Armada *et al.*, 2015). The AMF secrete phosphatase to hydrolyze phosphate from organic P compounds and thus improve the crop productivity under P-deficient conditions (Smith *et al.*, 2011). The mycorrhizal hyphae also increase the uptake of ammonium, immobile micronutrients (such as Cu and Zn), and other soil-derived mineral cations (K^+, Ca^{2+}, Mg^{2+}, and Fe^{3+}) (Smith and Read, 2008). The PGPR secrete compounds that increase the cell permeability and enhance the root exudation rate of plants (Nadeem *et al.*, 2014). Such nutritive exudates support dense fungal colonization and facilitate root penetration (Armada *et al.*, 2015). Fungi penetrate the plant roots, enhance the root surface area and boost the nutrient and water acquisition by plants (Barzana *et al.*, 2012). In addition, the PGPR expand mycosymbionts and facilitate the AMF colonization (Armada *et al.*, 2015). Thus, the PGPR not only support the synergistic mycorrhizal colonization, but also improve the additional functions of nutrient uptake. Two or more strains of various AMF as well as rhizobacterial strains impart their effects on nutrient uptake, plant health, and the expression of key transporter genes involved especially in N and P uptake. These associations have been studied on the soils with either low N availability (unfertilized plots) or on those with easily mineralizable organic fertilizer. Apart from N fertilization, inoculation with AMF alone or in combination with PGPR at the tillering stage increases aboveground biomass, which is due to the expression of nitrate transporter genes (Saia *et al.*, 2015). Plants inoculated with PGPR and AMF have been shown to absorb water and nutrients more efficiently under water-deficient environments (Ruíz-Sánchez *et al.*, 2011). This may be due to improved root architecture that results in better root growth with lateral root formation (Hodge and Storer, 2015). The PGPR-AMF interactions under extreme water regimes also affect the switching of water flow through the apoplastic or symplastic pathways and thus improve the stress tolerance of plants (Barzana *et al.*, 2012). Production of antibiotics against fungal plant pathogens (Castillo *et al.*, 2002) and synthesis of some bioactive compounds (Jansa *et al.*, 2013) are considered the other functional activities of PGPR-AMF interactions. The PGPR-EcMF interactions are also important for ecosystem functioning (Rincón *et al.*, 2005). Recently, Labbé *et al.* (2014) reported new mycorrhiza helper bacteria. They observed that *Pseudomonas* strains improve the root colonization and stimulate the ectomycorrhizal formation in *Populus deltoides* roots. Improved root colonization helps in the formation of ectomycorrhizal Hartig net (Henke *et al.*, 2015). With the help of such improved ectomycorrhizal networks, the exchange of water and nutrients between different individual trees can take place. This enhances the stability and fitness of forest ecosystems under adverse environments (Luo *et al.*, 2014). The rhizosphere PGPR-fungi interactions have been suggested as one of a previously unimagined complexity where PGPR and AMF positively interact to provide multifunctional benefits to improve plant health and soil fertility. The PGPR-AMF interactions improve rhizosphere functioning under stress conditions, and such associations mitigate the stress-induced effects on plants through various mechanisms and gene expressions.

PGPR-fungi associations in restoration of degraded land and soil conditioning

Intensive agricultural practices have deteriorated soil fertility and quality. According to an estimate, such agricultural practices will convert about 30% of the total world cultivated soils into degraded land by 2020 (Rashid *et al.*, 2016). Depletion of fertile soil is presently considered as one of the emerging issues owing to escalating global population, pollution, and perturbation of natural resources. Abiotic environmental factors and their variability are emerging as the major challenges facing the agriculture performance. In particular, reductions in the belowground microbial diversity and activity are usually associated with land degradation (Patel *et al.*, 2015; Singh, 2015a). Efficient microorganisms can significantly contribute to agriculture and environmental stability (Singh *et al.*, 2011). The influence of EPS-producing PGPR on the aggregation of root-adhering soils has been well described under different environmental stresses (Nunkaew *et al.*, 2015). The microbial EPS bind soil particles to form micro- and macroaggregates along with fungal hyphae and thus stabilize the soils (Grover *et al.*, 2011). The EPS-producing PGPR have been reported to significantly enhance the volume of soil macropores and rhizosphere soil aggregation, resulting in an increase in water and fertilizer availability to plants, which in turn helps the plants to better manage the adverse effects of salinity (Upadhyay *et al.*, 2011). Plants treated with EPS-producing bacteria exhibit increased resistance to water stress due to improved soil conditions (Sandhya *et al.*, 2009). The EPS from PGPR bind to the cations including Na^+, and it is expected that an increase in the population density of EPS-producing bacteria in the root zone would decrease the Na^+ availability to the plants and thus help in alleviating salt stress in saline environments (Nunkaew *et al.*, 2015). Phytohormones produced by PGPR are also effective in promoting the plant growth under stressed environments. Sadeghi *et al.* (2012) demonstrated that a *Streptomyces* strain improves the growth of wheat by producing plant growth-promoting hormones, indole acetic acids (IAA), and auxins under salt stress. The AMF develop intensively inside the roots and within the soil by forming an extensive extraradical network that helps the plants considerably in exploiting soil mineral nutrients and water (Ortiz *et al.*, 2015). Insoluble glomalins (glycoproteins) produced by AMF play an important role in the stabilization of micro- and macroaggregates and improve the soil structure and stability. The AMF also have the potential to influence C and N cycling in the alpine grasslands (Li *et al.*, 2015). The formation of a hyphal network around the root zone by mycorhizal fungi not only provides water and nutrients to the plants, but also filters heavy metals through biofiltration to restrict their availability to the plants (Miransari, 2011). Ectomycorrhizae have been found to be crucial for the restoration of forest ecosystem, while AMF for agriculture ecosystems. Co-inoculation of bacteria and fungi with or without organic fertilizer has been found to be more beneficial for reinstating the soil fertility and organic matter content than their single inoculation (Rashid *et al.*, 2016). Further investigations on the microbial interactions will help in reinstating the fertility of degraded soils and also in managing the agricultural soil N demands by antagonizing the negative impact of chemical fertilizers.

CONCLUSIONS

Stressful environments not only deteriorate soil structure, but also affect crop productivity. Increasing concerns for a safe environment and excessive and deliberate use of chemicals in the modern agriculture necessitate looking for the eco-friendly alternatives. The PGPR and AMF offer the attractive groups of beneficial microflora for sustainable agriculture, and have been of worldwide importance and gaining acceptance in stressed agriculture management and restoration of degraded lands. The application of such microbes not only improves soil physico-chemical properties, but also enhances plant growth on disturbed and stressed soils. Under stress conditions, plants produce stress-induced chemicals such as C_2H_4 that negatively affect plant growth. The PGPR have the ability to reduce the levels of stress-induced chemicals by producing enzymes such as the ACC deaminase and thus protect the plants against damages. The establishment of useful host defense enzyme sinks using PGPR community and the reduction in stress levels induce the elongation of roots, encourage the formation of branched roots, and minimize the hazardous effects of stress to promote the plant growth and viability. Furthermore, rhizobacteria play crucial roles in plant-microbe interactions owing to their ability to produce phytohormones and promote phytostimulation efficiency. The PGPR, a metabolically and functionally diverse group of soil-inhabiting bacteria, exhibit multiple mechanisms that suppress phytopathogens and promote crop growth. They benefit plants through various mechanisms such as competitive root colonization, phosphate solubilization, Fe sequestration, production of phytohormones, enhancement of nutrient uptake *via* mineral solubilization, and synthesis of anti-pathogenic lytic enzymes. Novel PGPR strains having multifunctional genetic configurations could be a potent tool for plants to cope up with the harsh environmental conditions. The AMF are a key component of soil microbiota that regulate plant growth and nutrient uptake and at the same time stabilize soil aggregates, making the soil less susceptible to erosion as well as degradation. Genomic profiles of naturally occurring PGPR and AMF open new vistas for improving rhizospheric microbial communications for the synthesis of host defence enzymes under stress conditions. A better understanding of the different mechanisms involved in plant-microbe and microbe-microbe interactions is a prerequisite for developing new strategies for improving crop yields. These beneficial microorganisms can be used as efficient bio-agents in the management of stressed agriculture.

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BOOK REVIEW
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Book Review: Microbial Inoculants in Sustainable Agricultural Productivity- Vol. II: Functional Application

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A Book Review on
Microbial Inoculants in Sustainable Agricultural Productivity- Vol. II: Functional Application

by D. P. Singh, H. B. Singh, and R. Prabha, (New Delhi; Heidelberg; New York, NY; Dordrecht; London: Springer), 2016, 308 pages, ISBN: 978-8132226420.

Ecologically sustainable agricultural practices are essential to ensure food security, and efficient agriculturally beneficial microbes (microbial inoculants) are playing potential role in sustainable crop production due to their immense plant growth promoting attributes, better adaptability to survival under stresses, and other uses that result in attenuating the pesticides/fertilizers use in agriculture. However, the unpredictable biogenic and abiogenic soil factors that determine the nature and magnitude of the microbial inoculants responses and survival after their delivery in the field conditions remain unresolved.

Soil microbes via several processes play indispensible roles in the supply of valuable nutrients to crop plants (Bashan et al., 2014). The presence of beneficial microbial communities in the rhizosphere minimizes the susceptibility to crop diseases (Singh, 2015). Because of ability to produce plant growth promoting and other molecules from secondary metabolism, beneficial microbes are widely used as commercial bio-inoculants (Singh et al., 2016a). However, a great diversity of valuable microbial inoculants continues to be revealed, and little is known about to the potential applications of new efficient microbial formulations that have been described.

The book *Microbial Inoculants in Sustainable Agricultural Productivity Vol. II Functional Applications* essentially addresses the field usage of microbial agents (biofertilizers, biostimulants, biopesticides) for boosting agriculture sustainability. In this volume, a total of 19 chapters have been distributed over 308 pages. It contains the relevant topics contributed by the well-known researchers from different universities and institutes. Entire chapters in different subject areas contributed by the leading authors can be grouped into four parts. The first part (chapter 1–11) highlights the use of bio-inoculants in management of crop plant stresses. This section provide satisfactory information about diverse group of microbes (rhizobia, cyanobacteria, actinomycetes, mycorrhiza, endophytes, etc.) that have been developed as microbial inoculants with beneficial functions at different levels and many chapters have touched on commercial production for applications in field conditions for farmers' benefits. Recently, plant growth promoting rhizobacteria (PGPRs) and cyanobacteria have gained attention for their indispensible role in restoration ecology and sustainable agriculture (Singh and Strong, 2016; Singh et al., 2016b). Therefore, selection of such efficient microbial strains with well-defined PGP mechanisms can be exploited in development of bio-fertilizer/bio-pesticide inoculants for achieving consistent and economical results under field conditions. Furthermore, these microbial inoculants with tested results can be considered as integrated nutrient management system to sustain agricultural productivity with no adverse environmental impact. Though, these bio-agents play a significant role in soil nutrient management, the knowledge about their roles, functioning and the mechanism

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of action is not completely understood under field conditions. Therefore, the performance of microbial inoculants under field conditions may be governed by more than one mechanism/factor and is a complex phenomenon that needs comprehensive study (Logue et al., 2015).

The second part of the book (chapter 12–15) is exclusively dedicated to explain about formulations, applications and delivery systems of microbial inoculants. Microbial bio-formulations commercially available and their mode of application in the field along with conventional methods of the delivery systems such as microbigation, seed bio-priming, seed encapsulation, fluid drilling, and consortia are also discussed. This volume confirmed that the common microbial agents used for the purpose of bio-inoculants production should have higher viable microbial count in field conditions, extended shelf life, efficiency, resistance to biotic and abiotic stresses, competence to indigenous soil micro-flora, affordability to farmers, and ability to create overall positive impacts on agriculture and environment.

The third part of the book (chapter 16–18) emphasizes about nanoparticles mediated novel technologies geared toward crop health. Nanoparticles based smart delivery system like nanosensors, nanofertilizers, nanopesticides, and nanoformulations to enhance agricultural health is also pointed out. Finally the last section of the book (chapter 19) highlights mainly on regulation and registration of bioinoculants. Registration requirement of bio-pesticides is mandatory to ensure safety to human health, benign effects to non-target organisms, and the environment. Although, in certain countries implemented regulations and framework indicated that use of bio-pesticides in agriculture is safe for human health, there are also some challenges and technical problems that need to be addressed through more effective policies and scientific approaches to enhance quality and safety of bio-pesticide.

The book lacks studies on impact of delivered microbial bio-inoculants on native microbial community structure and their interaction with soil and plants. Also, it is outside the purview of the book to review the methodologies which have been applied for monitoring the performance of introduced microbial inoculants in field conditions. It is evident from the investigations that the systems involving plant growth promotion by bio-agents include, in addition to the direct microbial consequences, their communication by signaling molecules with indigenous micro-flora, and the resulting impact on soil functioning. As most plant-inoculants interactions are as yet unexplored and high-throughput genomic and proteomic tools are limited, we can only predict that plant metabolite-mediated microbial inoculants differentiations could be general strategies of the plant-inoculants associations (Maróti and Kondorosi, 2014). Therefore, there is need to evaluate crucially such non-target effects of microbial inoculants at genomic, transcriptomic, and proteomic level, and to authenticate such results before their delivery in the natural field conditions. The information available in the book are quantitative with no attempt made to evaluate the impact of microbial inoculants on factors involved in N dynamics, an important driving force of soil, microbes, and plant sustenance. Nowadays bio-formulations (microbes or their metabolites) application has been considered as promising tools for sustainable crop production. Inoculation of beneficial microbes along with their metabolites may be a potential option, proving to be more valuable with multifarious roles and consequently indicating the new avenue to develop the bio-formulations for safe agriculture (Arora and Mishra, 2016). However, the eco-friendly viable bio-formulations development at commercial level and constraints associated with them are not discussed in this volume.

In conclusion, this volume offered detailed information on mass production of efficient microbial inoculants from laboratory to industrial/commercial level, intellectual property rights required, registration processes, bio-safety and bio-security concerns, quality control, and their legal authenticity. Based on the above merits, we recommend this book to stakeholders engaged in working on microbial bioinoculants.

AUTHOR CONTRIBUTIONS

All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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PRESENTATIONS

PRESENTATIONS IN CONFERENCE/WORKSHOP/SEMINAR/SYMPOSIUM

i. Vimal, S.R., Kumar, A., Tewari, S. Singh, J.S., 2014. “PGPR can be used as possible bio-agent to enhance agricultural production under various environmental stresses” in International conference on environmental technology and sustainable development; challenges and remedies (February, 21-23) 2014 held at Babasaheb Bhimrao Ambedkar University (A Central University)-Lucknow (INDIA).

ii. Vimal, S.R., Singh, J.S., 2014. “Eco safe PGPR for second green revolution” in National workshop on recent advances in PGPR research (October, 7-8) 2014 held at Banaras Hindu University-Varanasi (INDIA).

iii. Vimal, S.R., Singh, J.S., 2014. “Role of industries in commercialization of PGPR as bio-fertilizer” in National workshop on “Innovation and technology transfer to industries role of universities” (March, 10-11) 2014 held at Babasaheb Bhimrao Ambedkar University (A Central University)-Lucknow (INDIA).

iv. Vimal, S.R., Singh, J.S., 2015. “Emerging microbial bio-fertilizer technology for climate smart sustainable agriculture” in International workshop on “Bridging development divide for inclusive growth through science technology and innovation” (January, 16-17) 2015 held at Babasaheb Bhimrao Ambedkar University (A Central University)-Lucknow (INDIA).

v. Vimal, S.R., Singh, J.S., 2015. “PGPR can be used as safe, viable and green agriculture” in National Seminar on Recent trends in applied microbiology human health and environment (March, 27-28) 2015 held at Bundelkhand University-Jhansi (INDIA)

vi. Vimal, S.R., Singh, J.S., 2015. “Plant microbe manure association: a tripartite interaction in stress agriculture management” in National symposium on impact of climate change on plant microbe interactions and its implications (December, 18-19) 2015 held at Banaras Hindu University-Varanasi (INDIA).

vii. Vimal, S.R., Kumar, A., Singh, J.S., 2015. “Cyanobacteria : a green capsule for biofertilizer purposes” National workshop on “University industry partnership: a march towards sustainable growth” (March, 12-13) 2015 held at Babasaheb Bhimrao Ambedkar University (A Central University)-Lucknow (INDIA).

viii. Vimal, S.R., Singh, J.S., 2016. “Tuning PGPR services for restoration of tainted agro-ecosystem” in National conference on managing soil resource for environmental sustainability: challenges and perspectives (December, 9-10) 2016 held at Banaras Hindu University-Varanasi (INDIA).

ix. Vimal, S.R., Singh, J.S., 2017. “Exploiting plant-microbe partnerships for better crop plant productivity under soil salinity” in Lucknow science congress (March, 3-4) 2017 held at Babasaheb Bhimrao Ambedkar University (A Central university) -Lucknow (INDIA).

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