EVALUATION OF PERFORMANCE OF PLANT GROWTH PROMOTING BACTERIA (PGPB) CONTAINING 1-AMINOCYCLOPROPANE- 1-CARBOXYLATE DEAMINASE (ACCD) IN IMPROVING GROWTH AND YIELD OF CEREAL CROPS UNDER SALINITY STRESS

Thesis

Submitted in Partial Fulfillment of the Requirement for the Award of the Degree of

DOCTOR OF PHILOSOPHY

IN

MICROBIOLOGY

BY

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CERTIFICATE OF ORIGINAL WORK

This is to certify that the study conducted by **Ms. Alka Sagar, Id. No. 12PHCMB104,** during **2012-2017** as reported in the present thesis was under my guidance and supervision. The results reported by her are genuine and the candidate herself has written the script of the thesis. Her thesis entitled, "**Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) in improving growth and yield of cereal crops under salinity stress**", is therefore, being forwarded for the acceptance in partial fulfilment of the requirements for the award of the degree of **Doctor of Philosophy** in **Microbiology,** of the **Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering**, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad – 211007 (U.P).

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SELF DECLARATION

I, Alka Sagar declare that the work presented in this dissertation entitled **"Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) in improving growth and yield of cereal crops under salinity stress"** submitted to the Department of Industrial Microbiology, in the Faculty of Engineering and Technology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, for the award of the degree of Doctor of Philosophy in Microbiology is an original work. I have neither plagiarized nor submitted the same work for the award of any other degree. In case this undertaking is found incorrect, my degree may be withdrawn unconditionally by the University. .

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CERTIFICATE OF RECOMMENDATION BY STUDENT ADVISORY COMMITTEE

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ABBREVIATIONS

ACCD	1-aminocyclopropane-1-carboxylate Deaminase
BSA	Bovine Serum Albumin
^{0}C	Degree Celsius
cm	Centimeter
CAT	Catalase
cfu	Colony forming unit
D.W.	Distilled water
D.D.W	Double Distilled water
et al.	And co-workers
FCR	Folin–Ciocalteu reagent
Fig.	Figure
gm	Gram
GSH	Glutathione Reductase
Hrs.	Hours
HCN,	Hydrogen cyanide
IAA	Indole acetic acid
L	Litre
mg	Milli gram
ml	Milli litre
min.	Minutes
NA	Nutrient agar

PB	Phosphate Buffer
PBS	Phosphate Buffer Saline
PGPB	Plant growth promoting bacteria
PGP	Plant Growth Promoting
SHUATS	Sam Higginbottom University of Agriculture, Technology and Sciences
s	Second
sp.	Species
NH_3	Production of ammonia
PS	phosphorus solubilization activity
SD,	Siderophore
µg	Micro gram

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Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) in improving growth and yield of cereal crops under salinity stress

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ABSTRACT

Soil salinity is an important problem for crop production in many parts of the world, especially in irrigated fields of arid and semiarid regions. Plant growth promoting bacteria (PGPB) are known to reduce adverse effect of environmental stress and thereby enhance the growth and development of plant health, soil fertility and agricultural sustainability. PGPB contain enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) that inhibits the ethylene production from its precursor ACC into α-ketobutyrate and ammonia. Removal of ACC significantly increases plant root and shoot length and protect the plant from stress. In the present study, 650 bacterial cultures were isolated from the soil of Model Organic Farm (SMOF) of Sam Higginbottom University of Agriculture, Technology and Sciences (SHUATS), Allahabad, India. Rich bacterial diversity in soil from SMOF both in terms of their types and functional plant growth promoting (PGP) traits was observed and represented by heterotrophs, coliforms, *Pseudomonas* spp., *Azotobacter* spp. and *Rhizobium* spp. and majority of them displayed multiple plant growth promoting (MPGP) traits. Bacterial isolates were predominately positive to production of ammonia (NH_3) (93.2%), indole acetic acid (IAA) (89.6%), catalase (85.0%), 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) (78.6%) and siderophore (69.0%). Richness of their functional characteristics is further revealed by their tolerance to salinity and wide range of pH. In the present study all isolates from organic farm were tolerant to > 5 % NaCl and wide range of pH. We obtained major (96-97%) constituent of bacterial population of nitrogen fixers *Azotobacter* spp. and *Rhizobium* spp. with multiple PGP traits and tolerance to salinity and wide range of pH. Twenty nine (29) potential PGPB were identified on the basis of molecular with 16S rRNA and sequences were submitted to NCBI and got different accession no. Also the potential PGPB were tolerant to high levels of trace elements, and resistant to multiple antibiotics. On the basis of tolerance to high salt (20%) concentration

three salt tolerant PGPB (STPGPB) viz. *E. cloacae* (KP226569) (PR4), *Enterobacter* sp. (KP226570) (PR14) and *A. nigricans (*KP966496) (PR19) were selected for detailed studies. All the three *STPGPB* expressed significant increase ($p<0.001$) antioxidant enzymes activities (SOD, CAT and GSH) under the abiotic stress of salinity and pH. Inoculation with *STPGPB* exhibited higher percentage of seed germination and enhanced growth parameters in cereals seed such as rice, maize and millets under salinity stress. Further significantly ($p<0.001$) increased percentage of seed germination and enhanced growth of these cereals was noted under salinity in presence of ammonium sulfate (substitute of ACC) clearly demonstrating the role of ACCD in alleviation of abiotic stress, especially salinity.

Key words: Plant growth promoting bacteria (PGPB), 1-aminocyclopropane-1-carboxylate deaminase (ACCD), plant growth promoting (MPGP) traits, Cereals crops, Salinity stress

INTRODUCTION

Salinity is one of the major abiotic stresses that adversely affect modern agriculture and establishes a problem everywhere in the world. Salinity causes degradation of the physio-chemical properties of soil endangering the potential use of soils (**Ladeiro, 2012; Rengasamy, 2006**, resulting in major impacts on crop productivity (**Kumar *et al*., 2010**; **Tavakkoli *et al*., 2011**; **Chauhan *et al*., 2016**). Globally, approximately 1 billion ha of land (7% of all land area) are affected by soil salinity (**Yensen, 2008**). This is estimated to have a negative impact on one-third of the world's food production (**Munns, 2002**).

The largest areas of salt-affected soils are in Australia followed by North and Central Asia, South America and South and West Asia. In Asia alone, 21.5 million ha of land area is thought to be salt-affected, with India having 8.6 million ha of such area which constitutes a major part of problem soils in India (**Tomar, 2005**; **Sahi *et al*., 2006**), of which 2.5 M ha is in the Indo-Gangetic plain (**Saxena *et al*., 2004**; **Mandal and Sharma, 2006**; **National Remote Sensing Agency, 2008**; **Mandal and Sharma, 2011**). The problem is more pronounced in Haryana, Uttar Pradesh, Orissa, Gujarat, Punjab, Rajasthan, West Bengal, Maharashtra, Andra Pradesh, Karnataka and Tamil Nadu (**Dubey *et al*., 2007**).

Soil salinity is now considered as the frontier area of the Indian agricultural research. Adverse effects of salinity in plant are the results of complex interactions among morphological, physiological and biochemical processes including plant growth, water and nutrient uptake (**Bano and Fatima, 2009**; **Akbarimoghaddam *et al*., 2011**; **Haghighi and Pessarakli, 2013**; **Prasad *et al*., 2014**; **Liu and Zhang, 2015**), seed germination (**Zhang *et al*., 2010**; **Abari *et al*., 2011**; **Kaveh *et al*., 2011**) and at the molecular level (**Tester and Davenport, 2003**). Salinity causes nutritional disorders in plants which lied to deficiencies of several nutrients by drastically increasing intra and intercellular Na+ levels (**Ahmad *et al*., 2016**).

Soil salinity reduces crop growth and productivity due to reduction in respiration, and protein synthesis (**Ahmad and Prasad, 2011**). It also down regulates plant growth regulators synthesis and adversely affects root and shoot growth. (**Rajakumar, 2013**; **Radhakrishnan and Baek, 2017**). Crop yields start declining when pH of the soil solution exceeds 8.5 or EC value goes above 4 dS m-1.

Salinity severely affects photosynthesis mainly through 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**; **Haghighi and Pessarakli**, **2013; Liu and Zhang, 2015**). The most affected crops due to salinity in India are wheat (**Kalhoro *et al*., 2016**), bread wheat (**Mirzaei *et al*., 2012**), rice (**Joseph**, **2013**), oat (**Chauhan, 2016**) and foxtail millet (**Pandey *et al*., 2016)**.

Some artificial and mechanical methods that have been employed for removal of salt in soil include disposal of surface layers, use of electro-kinetic extraction, soil washing with clean water, or soil mixing with organic materials to improve soil structure (**AE 2001; USEPA 2000**). Unfortunately, these techniques are often impractical and costly as well as having other environmental drawbacks such as appropriate disposal of the contaminants.

Plant-growth-promoting bacteria (PGPB) are naturally present in the soil that insistently colonize plant root zone and promote plant growth through multiple mechanisms (**Patten and Glick, 2002**; **Vessey, 2003; Han and Lee, 2005**). The direct growth promoting mechanisms are i) nitrogen fixation ii) solubilization of phosphorus (PS) iii) sequestering of iron by production of siderophore (SD) iv) enhanced production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration (**Glick *et al*., 1999**). The indirect mechanisms of plant growth promotion by PGPB include i) antibiotic production ii) depletion of iron from the rhizosphere iii) synthesis of antifungal metabolites iv) production of fungal cell wall lysing enzymes v) HCN production vi) induced systemic resistance (**Kloepper *et al*., 1988**; **Liu *et al*., 1995; Glick *et al*., 1999**).

PGPB can improve plant growth via host plant resistance to biotic and abiotic stresses (**Kang *et al*., 2009**; **Richardson *et al*., 2009**; **Kang *et al*., 2014**). In addition, PGPB inoculation improves the plant growth and yield of various crops under normal and stress conditions (**Arruda *et al*., 2013**; **Barnawal *et al*., 2014**; **Martínez *et al*., 2015**).

These PGPB can produce bacterial exopolysaccharides (EPS) which bind in the root zone, decreased the Na^+ uptake and thus help in alleviating salt stress (**Han and Lee, 2005**). Inoculation of crop plants with certain strains of PGPB improves the percentage of seed germination (**Kaymak *et al*., 2008; Mishra *et al*., 2010**), plant vigor, chlorophyll content, yield, and nutrient uptake by variety of mechanisms under salt stress condition (**Saharan and**

Nehra, 2011).

PGPB protect plants from the adverse effects of soil salinity by reprogramming the stress induced physiological changes in plants. Bacteria can enrich the soil with major nutrients (nitrogen, phosphorus, and potassium) in a form easily available to plants and prevent the transport of excess sodium to roots (exopolysaccharides secreted by bacteria bind with sodium ions) for maintaining ionic balance and water potential in cells. (**Radhakrishnan and Baek, 2017**). Bacterial formulations have recently been used for the amelioration of salt stress in different crop plants (**Sarvanakumar and Samiyappan, 2007; Yue *et al*., 2007**). Plant-bacterial interaction reprograms the expression of salt stress-responsive genes and proteins in salinity-affected plants, resulting in a precise stress mitigation metabolism as a defense mechanism. Soil bacteria increase the fertility of soil and regulate the plant functions to prevent adverse effect of salinity in glycophytes (**Radhakrishnan and Baek, 2017**).

Salinity can induces the rate of ethylene biosynthesis *via* elevated levels of 1-aminocyclopropane-1-carboxylic acid (ACC) (**El-Beltagy *et al*., 1997**), which may lead to physiological alterations in plant tissues. Any check to this accelerated ethylene production in plants can improve growth of plants under salt stress. Recently it has been observed that some PGPB have the capability to produce 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) (EC 4.1.99.4), an enzyme which cleaves ACC, the immediate precursor of ethylene biosynthetic activity can in plants (**Glick *et al*., 1998**). Such bacteria which exhibit ACCD activity can indirectly inhibit the ethylene biosynthesis, therefore promoting the plant root growth and also protecting plant from stress (**Hontzeas *et al*. 2004**; **Belimov *et al*., 2005**; **Glick 2005; Glick *et al*., 2007**; **Hao *et al*., 2007**; **Farwell *et al*., 2007**; **Rodriguez *et al*., 2008**; **Gamalero and Glick, 2011**; **Etesami *et al*., 2015**; **Jha and Saraf, 2015**).

As a consequence, plant growth promoting bacteria exhibiting ACCD can protect plant from the deleterious effects of environmental stress. These bacteria might act as sink for ACC, thereby ameliorating some of the damage to plants caused by stress (**Mayak *et al*., 2004**; **Zahir *et al*., 2007**). PGPB containing ACCD decrease a significant portion of the physiological damage to plants by hydrolysing ACC, (the immediate precursor of ethylene synthesis) into ammonia and a-ketobutyrate (**Glick *et al*., 1999**). PGPB inoculation can suppress the adverse effect of stress in plants could be more eco-friendly, economical and easier option. In the same way, ACC deaminase-producing PGPB can protect plant from stress (**Glick, 2014; Mayak *et al*., 2004; Ali *et al*., 2012**).

Therefore, the present investigation has been carried out to enhance yield of cereal crops employing PGPB containing ACCD under salinity stress. Both lab and field work were conducted in this direction.

JUSTIFICATION

Soil salinity is destroying several hectares of arable land every minute and much greater agricultural area is at high risk of "transient salinity," significantly reducing agricultural crop production. At the same time, global food production needs to increase by approximately 50% by 2050 to match the projected population growth. Common crops including rice, wheat, maize, millets and sorghum are grown in wide range of environment and therefore, are exposed to various multiplicities and degrees of stresses. Salinity results in induction of multifaceted network of genes resulting in diverse degrees of complexities physiological responses in different crops. Many groups of scientist, globally, are working to discover sustainable solution against the salinity stress, but still it subsists as a challenge before the scientific community. Ethylene has long been regarded as a stress-related hormone and it may provoke programmed cell death, by inducing early senescence, or may impede growth by inhibiting root elongation and leaves growth, eventually damaging the plant's functional biology, ultimately having a negative impact of productivity. ACCD can inhibit ethylene production by hydrolyzing ACC (ethylene precursor) into ammonia and α-ketobutyrate. PGPB are group of bacteria, naturally habituating in rhizosphere and positively affecting the growth, physiology and above all yield of the plant by various means and also exhibit genes for ACCD production. Therefore, it is needed to explore natural micro-eco-systems to find novel PGPB that can antithesis adverse effects of high concentration of salt and retain crop productivity. The present study explores the screening of some potential PGPB with special reference to ACCD in improving growth and yield of cereal crops under salinity stress.

The present study was been planned with the following objectives:

1. To study the diversity and abundance of PGPB from soil of organic farm.
2. To develop a technology or strategy to enhance yield of cereal crops employing PGPB under salinity stress.
3. To evaluate the role of ACC Deaminase on the growth parameters and yield improvement of cereal crops under salinity stress.

REVIEW OF LITERATURE

2.1. Soil Salinity

Soil salinity is considered to be the most brutal environmental factor which caused a reduction in plant growth and crops productivity (**Allakhverdiev *et al.*, 2000**). Cultivated soils all over the world are becoming more saline due to continuing use of poor quality water for irrigation, excessive fertilization, and desertification processes (**Ramadoss *et al.*, 2013**). Salinity of arable lands is a major problem in agriculture. It causes a significant loss of crop productivity each year (**Francois *et al.*, 1994**). It is estimated that at least 20% of the irrigated lands worldwide are salt affected (**Qadir *et al*., 2014**). High salinity in agricultural land is a worldwide problem. High salt concentrations are plant growth inhibitory in many crops (**Cheng *et al.*, 2007**). High soil salinity adversely affects the physical and chemical properties of soil, thereby directly affecting the growth and diversity of organisms that live in or on soil such as plants, microbes, protozoa and nematodes (**Pitman and Läuchli, 2002; Parida and Das, 2005**) .

Salinity is one of the serious environmental problems that cause osmotic stress and reduction in plant growth, with adverse effects on germination, plant vigour and crop productivity in irrigated areas of arid and semiarid regions (**Munns and Tester, 2008**; **Hussain *et al.*, 2009**; **Shahbaz and Ashraf, 2013**). Soil salinisation is defined as process of increasing dissolved salts in the soil profile. It severely affects soil health (socio-economic wellbeing) which in turn affects crop productivity (**Rengasamy, 2006**). Salt stressed soils are known to suppress the growth of plants (**Paul, 2012**).

Soil salinity affects about 800 million hectares of arable lands worldwide (**Munns and Tester, 2008**). A soil is considered to be saline when the electric conductivity (EC) of the soil solution reaches 4 dS m^{-1} (equivalent to 40 mM NaCl), generating an osmotic pressure of about 0.2 MPa and significantly reducing the yields of most crops (**Munns and Tester, 2008**). As a consequence, ion toxicity, lead to chlorosis and necrosis, mainly due to Na+ accumulation that interferes with many physiological processes in plants (**Munns, 2002**).

Soil salinity has been reported to reduce yields, nodulation and the total nitrogen content in legume plants (**Singleton and Bohlool 1984**). **El-Fouly *et al.*, (2001)** found that the dry weight of different plant organs of tomato was reduced in response to the increase of NaCl level in the root growth medium.

Recently, there has been a great interest in eco-friendly and sustainable agriculture with emphasis on the use of beneficial microorganisms. Indeed, several recent studies have demonstrated that local adaptation of plants to their environment is driven by genetic differentiation in closely associated microbes (**Rodriguez and Redman, 2008**).

The research was conducted by the Institute for Water, Environment and Health during which it was found that for more than 20 years now, every day an average of 2,000 hectares of land is getting degraded by salt. The degraded land includes arid and semi-arid areas across 75 countries including, Aral Sea Basin in Central Asia, Indo-Gangetic Basin, Indus Basin of Pakistan, Chinese Yellow River Basin, Euphrates Basin, Australian Murray-Darling Basin and San Joaquin Valley in the United States. The total of global irrigated land covers some 310 million hectares and according to this research, salt-spoiled soil measures up to 20% of the total irrigated land (**Qadir *et al*., 2014**).

In the Indo-Gangetic Plain of Punjab, Haryana, Uttar Pradesh and Bihar saline sodic soils are usually found in high pH (9.0-10.2) high soluble salt content (EC in between 10 and 100 dSm-1) and high ESP> 40/SAR>13.The saline soils have high soluble salts (ECe> 4 dSm-1) of chlorides and sulphates of sodium, calcium and magnesium, low ESP (< 15) or low SAR (<13) and have pH values of less than 8.5. High osmotic pressure prevails in such soils which prevents absorption of water and nutrients by the plants (**Mandal *et al*., 2009**).

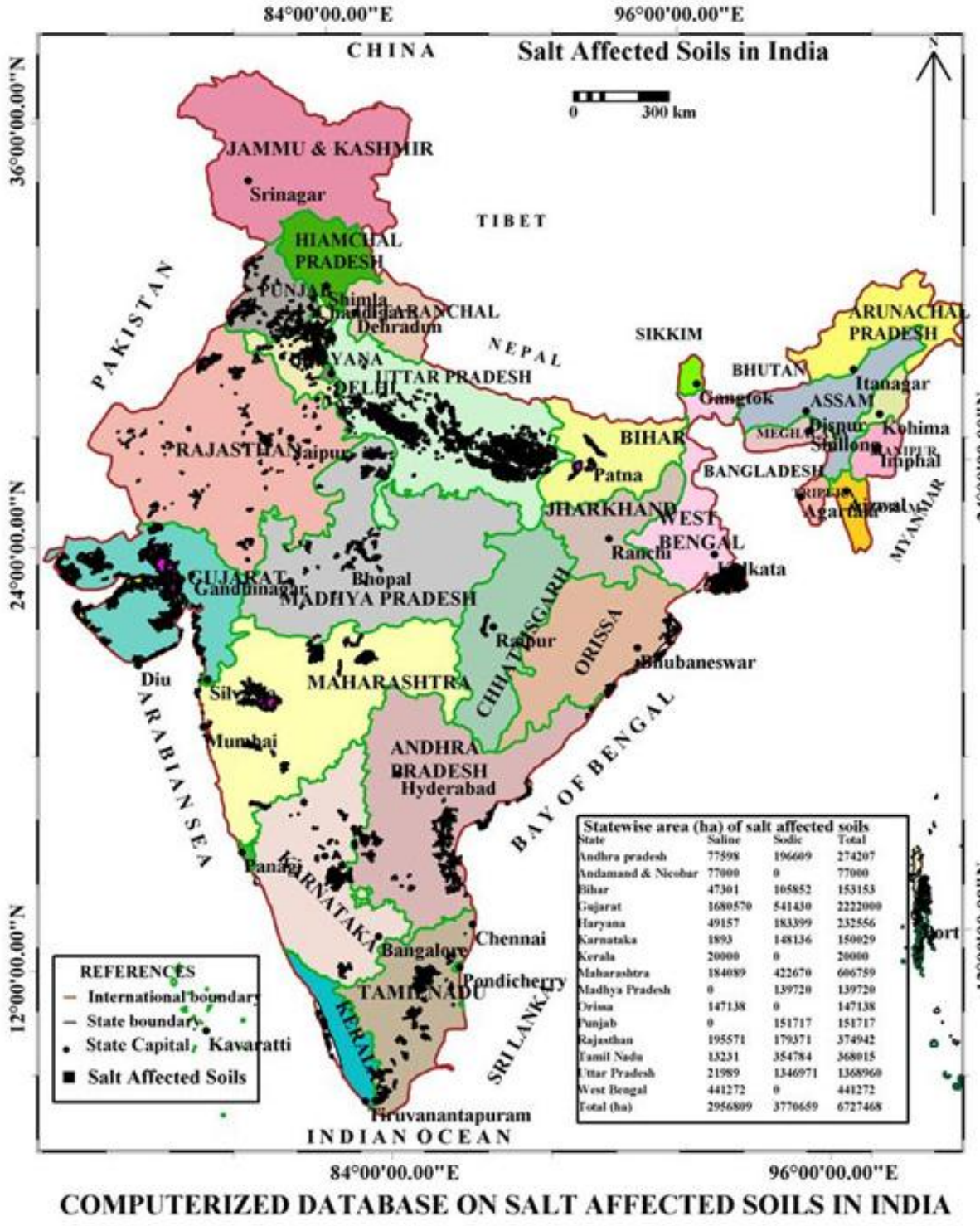


Figure 2.1. Distribution of salt-affected soils in India (Dagar *et al*., 2014)

2.1.1. Effect of salinity on plant growth

Excessive salt concentration augments the Na^+ and Cl^- ions level in different plants that negatively affect the plant survival by disrupting different plant metabolisms, cellular homeostasis, and uncoupling major biochemical as well as physiological processes (**Mahajan and Tuteja, 2005**). Although both Na+ and Cl^- ions have the capability to induce many physiological and/biochemical disorders in plants, nevertheless, Cl^- is among the most hazardous (**Tavakkoli *et al.*, 2011**). Salinity has negative impacts on plant growth and development (**Chinnusamy *et al.*, 2006**). The two toxic ions Na+ and Cl^- derived from NaCl can cause damage to plant cells through the induction of oxidative stress and osmotic stress, ion toxicity and nutrient deficiency (**Gao *et al.*, 2007**; **Talei *et al.*, 2015**).

Soils with salinity contain an array of several cation anion pairs (i.e., $CaSO_4$, $MgSO_4$, $MgCl_2$, Na_2CO_3, and Na_2SO_4), with Na+ ions being the predominant ion species (**Zhang *et al.*, 2010a**). Growth is impeding, a common plant response to salt stress is often related to elevated intracellular Na+ concentration and low K+/Na+ ratio in the plant (**Zhang *et al.*, 2010a**). Researchers have reported that plants grown under saline conditions can curtail sodium toxicity by limiting Na+ uptake, re-directing Na+ from shoots to roots, and also extruding Na+ loadings from root cells (**Munns and Tester, 2008; Zhang *et al.*, 2010a**, **2011**; **Kronzucker and Britto, 2011**). Further, the beneficial properties of microbes under salinity has been attributed to the hydraulic conductivity, sequestering toxic Na+ ions, osmolyte accumulation, retaining higher stomatal conductance and photosynthetic activities (**Dodd and Pérez-Alfocea, 2012**).

Adverse effect of salinity into complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, and water and nutrient uptake (**Akbarimoghaddam *et al.*, 2011**; **Chen *et al.*, 2013**; **Abbasi *et al.*, 2015**). At higher salinity, the expansion rate of the leaf area reduces together with a decrease in leaf production rate leading to the death of the plant (**Suarez and Medina, 2005**). It has been reported that increasing the salt level, reduces the osmotic potential, resulting cell dehydration, due to increased water efflux from cell (**Amjad *et al.*, 2014**; **Shrivastava and Kumar, 2015**).

Soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on plants, and thus limits water uptake from soil (**Bano and Fatima, 2009**;

Zhang *et al.*, 2010; **Tavakkoli *et al.*, 2011**). Salinity also severely affects growth, leaf coloration, anatomical potions, protein synthesis, lipid metabolism and photosynthesis (**Tester and Davenport, 2003**; **Parida and Das, 2005**; **Yildirim *et al.*, 2006**). **Acosta-Motos *et al.*, 2017** demonstrated that salt-induced oxidative stress at the subcellular level and its effect on the antioxidant machinery in both salt-tolerant and salt-sensitive plants.

2.1.2. Effect of salinity on soil characteristics

Salinity not only decreases the agricultural production of most crops, but also, effects soil physicochemical properties, and ecological balance of the area. The impacts of salinity include low agricultural productivity, low economic returns and soil erosions, (**Hu and Schmidhalter, 2002**). Soil salinity significantly reduces plant phosphorus (P) uptake because phosphate ions precipitate with Ca ions (**Bano and Fatima, 2009**).

2.2. Plant growth promoting bacteria (PGPB)

The narrow zone of soil, directly surrounding the root system, is referred to as rhizosphere (**Walker *et al.*, 2003; Lugtenberg and Kamilova, 2009**). Understanding how plant roots select soil microbes to form the microbial community of the rhizosphere is an important scientific issue when considering the use of rhizobacteria as plant growth promoters (**Drogue *et al.*, 2012**). While the term 'rhizobacteria' implies a group of rhizosphere bacteria competent in colonizing the root environment (**Kloepper *et al.*, 1991**), therefore, the rhizobacteria are the dominant deriving forces in recycling the soil nutrients and consequently, they are crucial for soil fertility (**Glick, 2012**). About 2–5 % of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed as PGPB (**Dimkpa *et al.*, 2009)**. PGPB were first defined by **Kloepper and Schroth (1978**), as a diverse group of bacteria that possess the remarkable capability to promote growth and yield of many crops and wild plants (**de-Bashan *et al.*, 2012**). These beneficial microorganisms colonize the rhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms (**Lugtenberg and Kamilova, 2009**; **Nia *et al.*, 2012**; **Ahmad *et al.*, 2013**; **Ramadoss *et al.*, 2013**).

One potential way to decrease negative environmental impact resulting from continued use of chemical fertilizers, herbicides and pesticides is the use of PGPB. They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting

plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds (like pesticides) (**Braud *et al.*, 2009; Hayat *et al.*, 2010**; **Rajkumar *et al.*, 2010; Ahemad, 2012**; **Ahemad and Malik, 2011**). The use of PGPB for reducing chemical inputs in agriculture is a potentially important issue. PGPB have been applied to various crops to enhance growth, seed emergence and crop yield, and some have been commercialized (**Dey *et al.*, 2004**; **Ashrafuzzaman *et al.*, 2009)**.

2.2.1. PGP traits

Many mechanisms have been reported for the activities of PGPB (**Glick *et al.*, 2007**). PGPB can stimulate plant growth directly by production of phytohormones (**Cassán *et al.*, 2009**; **Rashedul *et al.*, 2009**; **Abbasi *et al.*, 2011**) such as auxins (**Khalid *et al.*, 2004**); siderophores (**Filippi *et al.*, 2011**; **Yu *et al.*, 2011; Peek et al. 2012; Kannahi and Senbagam 2014**); phosphorous solubilization (**Yasmin *et al.*, 2004**; **Tajini *et al.*, 2012**; **Krey *et al.*, 2013**), or nitrogen-fixing (**Peix *et al.*, 2001; Riggs *et al.*, 2001**; **Fischer *et al.*, 2007; Navarro-Noya et al., 2012; Navarro-noya and Mart, 2013**) and lowering of ethylene (**Saleem *et al.*, 2007**) levels in plants through the synthesis of the enzyme 1-amino-cyclopropane- 1-carboxylate (ACC) deaminase that hydrolyzes the ethylene precursor ACC is another well-reported mechanism for growth promotion by PGPB (**Glick *et al.*, 2007**; **Shaharoona *et al.*, 2007**; **Saraf *et al.*, 2010**) and indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents (**Glick, 2012**) such as hydrogen cyanide (HCN), 2, 4- diacetylphloroglucinol (DAPG) (**Duffy *et al.*, 2004**); antibiotics, e.g., phenazine antibiotics (**Chakraborty *et al.*, 2009**); and volatile compounds that stimulate plant growth (**Ryu *et al.*, 2003**). **Rajput *et al.*, 2013** described that rhizobacteria improve plant growth employing a variety of growth promoting mechanisms including nutrient up take, root growth, proliferation and biocontrol activities.

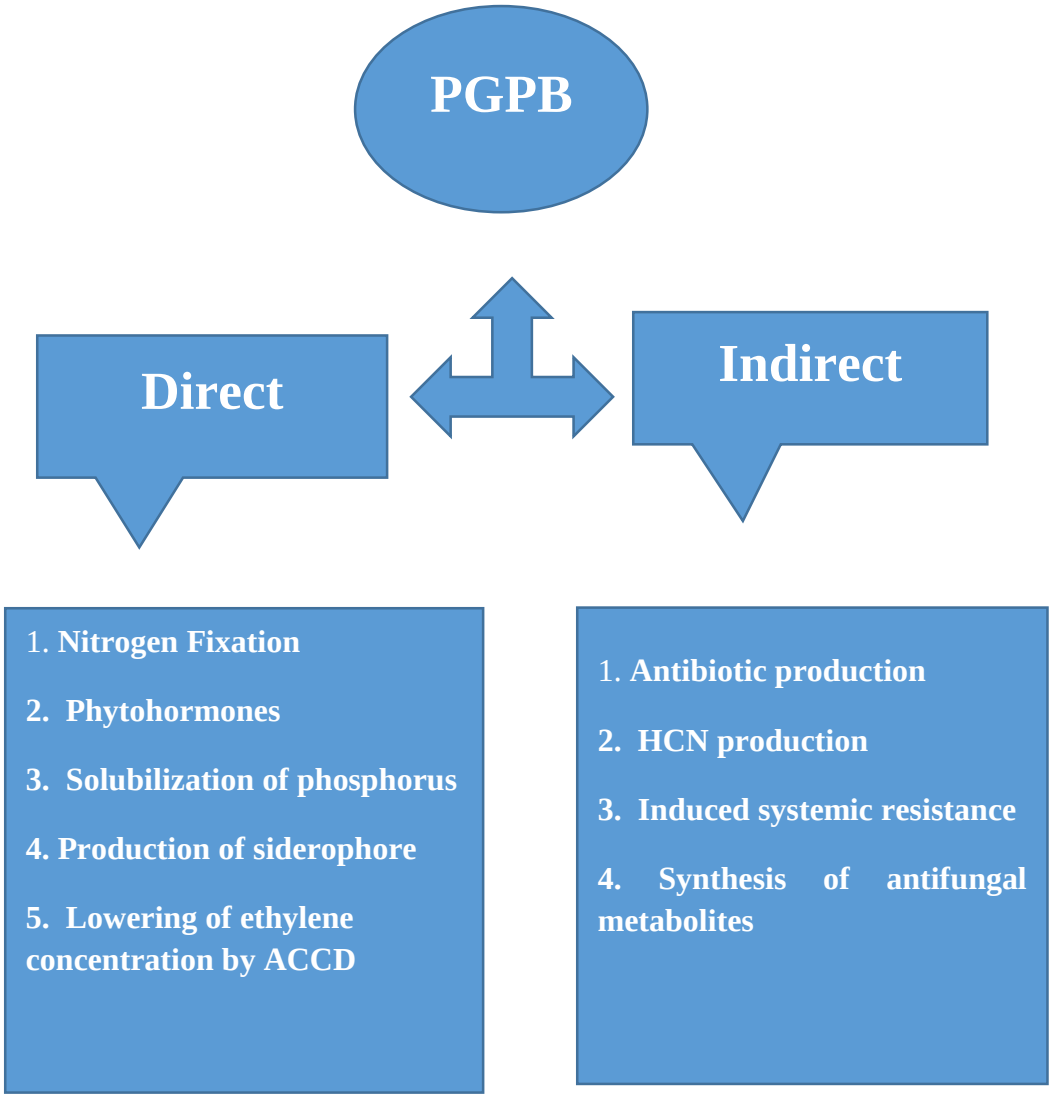


Figure 2.2. Diagrammatical representation of PGP traits

2.2.2. Classification of PGPB

PGPB may be classified into extracellular (ePGPB), existing in the rhizosphere, on the rhizoplane, or in the spaces between cells of the root cortex, and intracellular (iPGPB), which exist inside root cells, generally in specialized nodular structures (**Figueiredo *et al.*, 2011; Bruto *et al.*, 2014**).

2.2.2.1. Extracellular PGPB (ePGPB)

Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas and *Serratia* e (**Figueiredo *et al.*, 2011**; **Bhattacharyya and Jha, 2012)**

2.2.2.2. Intracellular PGPB (iPGPB)

Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium and Rhizobium of the family Rhizobiaceae (**Figueiredo *et al.*, 2011**; **Verma *et al.*, 2010).**

2.2.2.3. Other PGPB

Most of rhizobacteria belonging to gram-negative rods and gram-positive rods, cocci or pleomorphic (**Karlidag *et al.*, 2011**; **Yildirim *et al.*, 2011**; **Bhattacharyya and Jha, 2012**; **Desale *et al.*, 2014**).

2.2.3. Role of PGPB in enhancement of crop production and alleviation of salinity

PGPB have been used worldwide for many years as biofertilizers owing to their remarkable positive effects on crop yield and a range of biotic and abiotic stress tolerance (**Lugtenberg and Kamilova, 2009**; **Mayak *et al.*, 2004**; **Upadhyay *et al.*, 2012**). There are many evidences that PGPB improve plant salt tolerance by altering the endogenous hormone status (**Kang *et al.*, 2014**; **Sahoo *et al.*, 2014**;; **Upadhyay and Singh, 2015**; **Metwali *et al.*, 2015**; **Qin *et al.*, 2016**; **Kumar *et al.*, 2017**).

There are a number of reports that demonstrate the efficacy of PGPB for promoting plant growth under both normal conditions as well as in saline soils and other environmental stresses (**Egamberdieva 2009**; **Zahir *et al.*, 2009**; **Glick *et al.*, 2007**; **Karlidag *et al.*, 2011; Saharan and Nehra 2011**; **Yildirim *et al.*, 2011; Nadeem *et al.*, 2012**; **Mapelli *et al*.2013**; **Desale *et al*.2014**; **Qin *et al.*, (2014).** There is clear evidence that a diverse group of root-associated microbes is essential for promoting plant adaptation to salinity (**Munns and Gilliham, 2015**; **Tkacz and Poole, 2015**; **Turner *et al.*, 2013**; **De-Zelicourt *et al.*, 2013**)

Table 2.2. Role of PGPB in enhancement of crop production

PGPB	Crop	Response	Reference
Pseudomonas fluorescens and *Paenibacillus polymyxa*	Rice	reducing and non-reducing sugars, total phenol content and peroxidase (PO), polyphenol oxidase (PPO)	**Umashankari and Sekar, 2011**
Agrobacterium, Burkholderia, Enterobacter, and *Pseudomonas*	Rice	significantly enhances rice production	**De souza *et al.*, 2013**
Azospirillum brasilense, Azospirillum lipoferum and *Pseudomonas*	Rice	increase in root area, root length, number of tillers, straw and grain yields and total weight of plant	**Midrarullah *et al.*, 2014**
Pseudomonas putida, Pseudomonas fluorescens and *Azospirillum lipoferum*	Rice	improved plant growth and higher photosynthetic, improve the health and yield of the plants	**Sharma *et al.*, 2014**
Azospirillum brasilense	Wheat	Growth improvement	**Pereyra *et al.*, 2009**
AMF P. jessenii, P. synxantha	Wheat	Increased grain yield	**Mäder *et al.*, 2011**

Providencia sp. *Anabaena* sp. *Calothrix* sp.	Wheat	Enhancement protein content	**Rana *et al.*, 2012**
Serratia liquefaciens, Bacillus sp. *Pseudomonas* sp.	Maize	Increase yield (dry weight)	**Lalande *et al*., 1989**
Azospirillum brasilense, Brayrhizobium japonicum	Maize	Promote seed germination and early seedling growth in corn (Zea mays L.)	**Cassán *et al*., 2009**
Pseudomonas sp. and *Brevibacillus* sp.	Maize	Influenced by plant development	**Piromyou *et al.*, 2011**
Azospirillum, Pseudomonas	Maize	Promote maize seed germination, biomass and crop yield.	**Noumavo *et al.*, 2013**
Bacillus sp.	Barley	Increase root and shoot	**Çakmakçi *et al.*, 2007**
Azospirillum brasilense, Brayrhizobium japonicum	Soybean	Promote seed germination and early seedling growth in crop	**Cassán et al. 2009**
Sphingomonas sp, Bacillus subtilis, Serratia marcescens	Soybean	Improved physiological characteristics such as shoot/root length, fresh/dry weight, and chlorophyll contents	**Asaf *et al.*, 2017**
Bacillus subtilis, B. amyloliquefaciens, B. licheniformis, Pseudomonas fluorescens and *Chryseobacterium balustinum,*	Tomato	Significantly higher percentages of healthy plants	**Domenech *et al.*, 2006**

Bacillus subtilis, B. amyloliquefaciens, B. licheniformis, Pseudomonas fluorescens and *Chryseobacterium balustinum,*	Pepper	Significantly higher percentages of healthy plants	**Domenech *et al.*, 2006**
Bacillus subtilis	Tomato	Significantly enhanced plant height, fresh weight, number of fruits per plant and average weight of fruit compared	**Hariprasad *et al.*, 2011**
B. amyloliquefaciens, B. pumilus, B. subtilis	Tomato	Increase root to shoot	**Myresiotis *et al.*, 2014**
Bacillus circulans	Tomato	Increase in seed germination , shoot length, root length shoot dry weight, root dry weight,	**Mehta *et al.*, 2015**
Bacillus pumilus	Tomato	Significantly improved the shoot fresh weight and dry weigh	**Khan *et al.*, 2016**
B. thuringiensis	Tomato	Seed germination and shoot elongation	**Qi *et al.*, 2016**
Pseudomonas jessenii and *Pseudomonas synxantha*	Tomato	Significantly enhanced plant growth	**Sharma and Sharma, 2017**

Table 2.3. Role of PGPB in enhancement of crop improvement under salinity stress

PGPB	**Crop**	**Response**	**Reference**
Salt tolerant bacteria	Rice	Increased fresh and dry weight of shoot and root, chlorophyll a, b and cartenoid contents	**Nadeem *et al.*, 2006**
P.fluorescens, Chryseobacterium balustinum	Rice	Protected rice against rice blast and increase productivity and quality	**Lucas *et al*., 2009**
Pseudomonas extremorientalis and *P. chlororaphis*	Rice	Increased root and shoot length	**Egamberdieva, 2011**
Bacillusamyloliquefaciens	Rice	Increased plant growth	**Nautiyal *et al.*, 2013**
Thalassobacillus denorans and *Oceanobacillus kapialis*	Rice	Increase in germination percentage and rate	**Shah *et al.*, 2017**
Glomus sp., *B. circulans* and *Cladosporium herbarum*	Wheat	Increases dry weight , grain yield	**Singh and Kapoor 1999**
Bacillus subtilis and *Arthrobacter sp*	Wheat	Increase in dry biomass, total soluble sugars and proline content	**Upadhyay *et al.*, 2012**

Planococcus rifietoensis	Wheat	Enhanced growth and yield	**Rajput *et al.*, 2013**
Halotolerant and Halophilic bacteria	Wheat	Increased the root and shoot length and total fresh weight	**Orhan, 2016**
Enterobacter cloacae	Wheat	Increase in growth parameters, biomass, and chlorophyll content	**Singh *et al.*, 2017**
Klebsiella sp.	Wheat	Increase in proline, total soluble sugar, and total protein content of treated plants	**Singh and Jha, 2017**
Staphylococcus sciuri	Maize	Significantly accumulated nutrients in roots and shoots, and enhanced chlorophyll and protein contents in	**Akram *et al.*, 2016**
Phosphate solubilizing bacteria	Maize	Increased seed germination, plant´s growth and P content	**Anzuay *et al.*, 2017**
Curtobacterium flaccumfaciens	Barley	Increase in growth	**Cardinale *et al.*, 2015**
Phosphate solubilizing baceria	Peanut	Increased seed germination, plant´s growth and P content	**Anzuay *et al.*, 2017**
P. aeruginosa, *P. stutzeri*	Tomato	Enhancement in root and shoot length	**Tank and Saraf, 2010**

2.3. Ethylene production

Ethylene the simplest gaseous plant growth regulator (PGR), controls diverse physiological pathways in plants. Under various stress conditions and during different developmental stages, such as fruit ripening, seed germination, root elongation, leaf and flower senescence, seed germination, tissue differentiation and organ abscission, ethylene biosynthesis is significantly increased. (**Bleecker and Kende, 2000**; **Belimov *et al.*, 2001**; **Saravanakumar and Samiyappan, 2007**; **Ju and Chang, 2015**; **Schaller and Voesenek, 2015**; **Abiri *et al.*, 2017**).Ethylene acts as negative plant growth regulator as its concentration increases than required level under stressful environment (**Holguin and Glick, 2001; Huang *et al.*, 2003**). It may be possible that ethylene plays a small, negative role in the crop response to salinity stress, at least during a certain growth stage (**Yang *et al.*, 2009**; **Tao *et al.*, 2015**).Salinity stress enhanced ethylene level in the root rhizosphere leading to physiological changes in leaf tissues (**Wang *et al.*, 2002**; **Zapata *et al.*, 2004**; **Tank and Sarf, 2010**). Nonetheless, several other studies have reported that ACC may negatively regulate seedling growth in some plants under salinity stress (**Albacete *et al.*, 2009**). Salinity stress increased ACC level and as a result more ethylene (C_2H_4) production that ultimately increases plant damage (**Kukreja *et al.*, 2005**). 1-aminocyclopropane-1-carboxylate (ACC) is immediate precursor of ethylene. Etiolated pea seedlings demonstrate a characteristic classical ‘‘triple’’ response to ethylene (**Arshad and Frankerberger, 1988**). It involves reduction of stem elongation, swelling of hypocotyl, and change in the direction of growth. These findings further suggest that crops grown under salinity stress tend to synthesise an increased amount of ethylene (**Chen *et al.*, 2014**). In addition, the combined application of ET with other hormones, such as cytokinin and auxin, allows for regulation of the ethylene level and further enhances agricultural production (**Müller and Munné- Bosch, 2011; Bakshi *et al.*, 2015**).

2.4. ACCD

PGPB having enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase (E.C. 4.1.99.4) promotes plant growth by lowering plant ethylene levels due to salinity. ACC deaminase activity hydrolysis ACC into ammonia and α- ketobutyrate for use as a nitrogen and energy (**Mayak *et al.*, 2004**; **Glick *et al.*, 2007**) and promote the plant growth under saline environment (**Nadeem *et al.*, 2010**; **Siddkee *et al.*, 2010**). In addition, several forms of stress

are relieved by ACC deaminase producers, such as effects on phytopathogenic bacteria, and resistance to stress from polyaromatic hydrocarbons, and from salt and draught (**Penrose *et al.*, 2001**; **Glick, 2005**; **Glick *et al.*, 2007**; **Sun *et al.*, 2009**; **Shaharoona *et al.*, 2011**; **Rashid *et al.*, 2012**; **Nadeem *et al.*, 2010**; **Deepti *et al.*, 2014**). The *acdS* gene, encoding ACC deaminase, has been isolated from different species and polymerase chain reaction-based screening for the acdS and a reliable colorimetric ninhydrin assay have been recently developed (**Li *et al.*, 2011; Nikolic *et al.*, 2011; Jasim *et al.*, 2015**).

2.4.1 Characteristics of ACCD

ACCD is a multimeric enzyme with a monomeric subunit molecular mass of approximately 35– 42 kDa. It is a sulfhydryl enzyme that utilizes pyridoxal 5-phosphate as an essential co-factor (**Glick *et al.*, 1998**).) Pyridoxal phosphate is tightly bound to the enzyme in the amount of approximately one molecule per subunit; it displays a characteristic pyridoxaldimine visible absorbance at 418 nm. While several D-amino acids, notably D-serine and D-cysteine can act as substrates for ACC deaminase (albeit less efficiently than ACC), L-serine and L-alanine are effective competitive inhibitors of the enzyme (**Yao et al., 2000**). Despite the fact that its substrate ACC is plantproduced, in those instances where it has been examined ACC deaminase is not a secreted enzyme. Rather, it is localized within the cytoplasm of the microorganism that produces it.

2.4.2. Mechanism of ACCD Action

A model is proposed to explain how ACC deaminase-containing plant growth promoting bacteria can lower plant ethylene levels and in turn stimulate plant growth (**Glick *et al.*, 1998**), especially under stress conditions. In this model, the plant growth-promoting bacteria bind to the surface of either the seed or root of a developing plant; in response to tryptophan and other small molecules in the seed or root exudates, the plant growth promoting bacteria synthesize and secrete the auxin, Indoleacetic acid (IAA), some of which is taken up by the plant. This IAA together with endogenous plant IAA can stimulate plant cell proliferation and elongation, or it can induce the activity of ACC synthase to produce ACC (**Penrose and Glick 2001**). Some of the plant's ACC will be exuded along with other small molecules such as sugars, organic acids and amino acids. The exudates may be taken up by the bacteria and utilized as a

food source of the rhizosphere bacteria. ACC may be exuded together with the other components of the root or seed exudates. ACC may be cleaved by ACC deaminase to form ammonia and α-ketobutyrate, compounds that are readily further metabolized by the bacteria. The presence of the bacteria induces the plant to synthesize more ACC than it would otherwise need and also, stimulates the exudation of ACC from the plant (some of which may occur as a consequence of plant cell wall loosening caused by bacterial IAA). Thus, plant growth promoting bacteria are supplied with a unique source of nitrogen in the form of ACC that enables them to proliferate/survive under conditions in which other soil bacteria may not readily flourish. And, as a result of acting as a sink for ACC and lowering its level within the plant, the amount of ethylene that is produced by the plant is also reduced. Thus, the inhibition of plant growth by ethylene (especially during periods of stress) is decreased and these plants generally have longer roots and shoots and greater biomass.

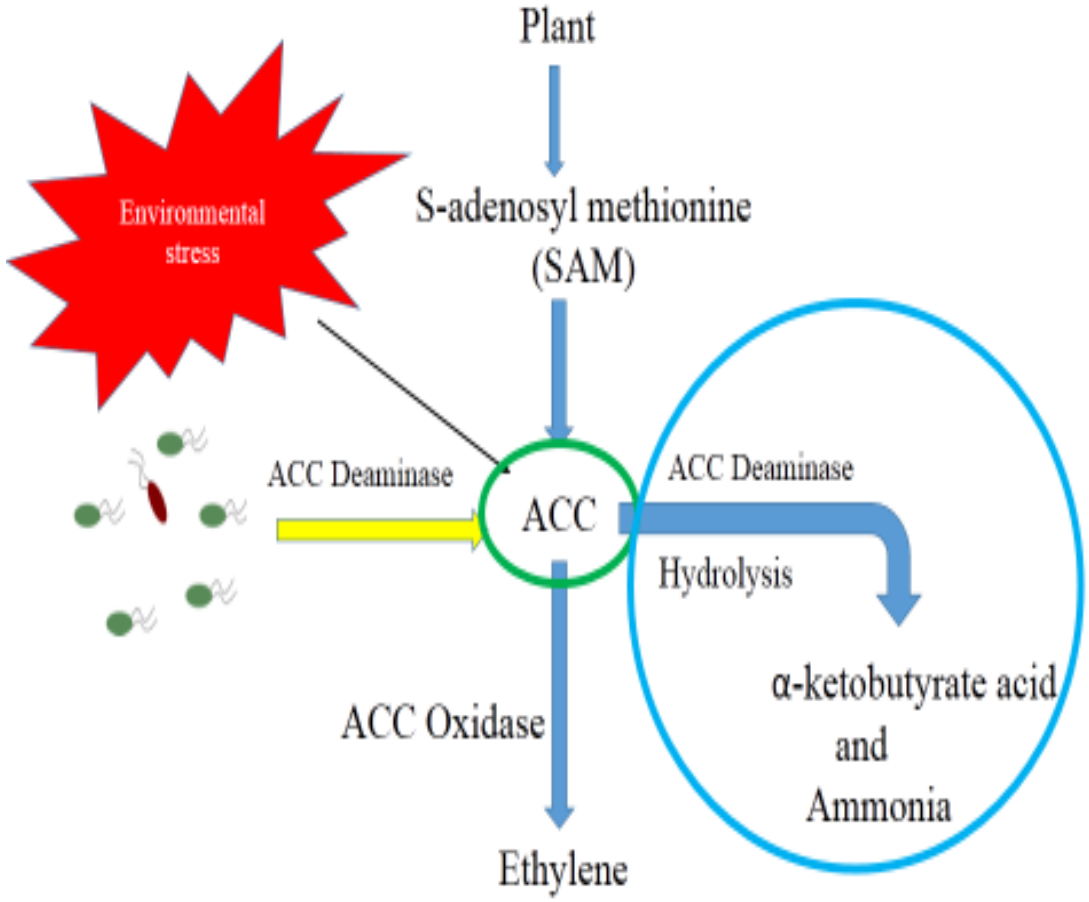


Figure 2.3. Diagrammatical representation of action of ACCD

Table 2.4. List of ACCD producing bacteria

ACCD producing bacteria	**References**
Gram negative	**Wang *et al.*, 2000**; **Babalola *et al.*, 2003**
Gram positive	**Belimov *et al.*, 2001**, **Ghosh *et al.*, 2003**
Alcaligenes	**Belimov *et al.*, 2001**
Bacillus sp.	**Belimov *et al.*, 2001**, **Woitke *et al.*, 2004**
Variovorax paradoxus	**Belimov *et al.*, 2001**
Rhizobia	**Ma *et al.*, 2003**; **Uchiumi *et al.*, 2004**
Pseudomonas	**Hontzeas *et al.*, 2004**; **Blaha *et al.*, 2006**; **Indiragandhi *et al.*, 2008**; **Rajkumar and Freitas 2008**; **Sahay *et al.*, 2012**
Burkholderia	**Blaha *et al.*, 2006; Jiang *et al*., 2008**
endophytes	**Pandey *et al.*, 2005**, **Sessitsch *et al.*, 2002**
Sinorhizobium meliloti	**Belimov *et al.*, 2005**
Ralstonia solanacearum	**Blaha *et al.*, 2006**
Rhodococcus	**Stiens *et al.*, 2006**
Agrobacterium genomovars and *Azospirillum lipoferum*	**Blaha *et al.*, 2006**
Methylobacterium fujisawaense	**Madhaiyan *et al.*, 2006**
Enterobacter sp	**Nadeem *et al.*, 2007**; **Kumar *et al.*, 2008**, **Habib *et al.*, 2016**
P. *fluorescens*	**Kumar and Samiyappan 2007; Nadeem *et al.*, 2007; Kumar *et al.*, 2008**; **Ahmad *et al.*, 2011**; **Ali *et al.*, 2014**

Pseudomonas syringae	**Nadeem *et al.*, 2007, Ahmad *et al.*, 2011**
Acinetobacter sp	**Shaharoona *et al.*, 2008**
Burkholderia	**Jiang *et al.*, 2008**
Serratia marcescens	**Poonguzhali *et al.*, 2008**
B. marisflavi, B. baekryungensis, B. selenatarsenatis	**Sahay *et al.*, 2012**
B. *Zhihengliuella alba, B. iodinum*.	**Siddikee *et al.*, 2012**
Bacillus licheniformis	**Sahay *et al.*, 2012**; **Siddikee *et al.*, 2012**,***Singh and Jha* 2016**
Halomonas salina	**Sahay *et al.*, 2012**
Oceanobacillus picturae	**Sahay *et al.*, 2012**
Thalassobacillus devorans	**Sahay *et al.*, 2012**
Arthrobacter protophormiae	**Barnawal *et al.*, 2014**
Microbacterium sp	**Shrivasta and Kumar 2014**
P. migulae	**Ali *et al.*, 2014**

2.4.3. Role of ACCD in alleviation of plant stress

ACC deaminase PGPB boost plant growth particularly under stressed conditions by the regulation of accelerated ethylene production in response to a salinity stress. Applications of PGPB containing ACC deaminase in relation to the nature of stress are described below.

Table 2.5. Plant response to inoculation with rhizobacteria containing ACC-deaminase under normal conditions

PGPB	**Crop**	**Response**	**Reference**
Rhizobium leguminosa- rum bv. Trifolii SN10	Rice	promote the growth of biomass, root branching and N content	**Bhattacharjee *et al.*, 2012**
Pseudomoas spp. and *Burkholderia caryophylli*	Wheat	Significant increase in root elongation, root weight, tillers plant−1, 1000-grain weight, grain and straw yield	**Shaharoona *et al.*, 2007b**
P. *fluorescens*	Wheat	Increased the growth and yield of wheat under fertilized conditions.	**Naveed *et al.*, 2008**
Pseudomonas spp.	Wheat	Seed inoculation enhanced growth, yield and nutrient use efficiency both under potted and field conditions at graded fertilizer levels	**Shaharoona *et al.*, 2008**
Bacillus spp	Wheat	Improving growth, yield	**Baig *et al.*, 2012**
Enterobacter cloacae and *Citrobacter* sp.	Wheat	Inoculated wheat seedlings showed notable increases in fresh and dry biomass	**Mishra *et al.*, 2017**

Pseudomonas fluorescen	Maize	Inoculation significantly improved the growth and yield	**Shaharoona et al. 2006a**
P. *putida* biotype A, P. *fluorescens* biotype G	Maize	Root-shoot length and seedling weight significantly increased by inoculation.	**Shaharoona et al. 2006b**
Bradyrhizobium sp.	Mung bean	Combined inoculation enhanced the growth and nodulation.	**Shaharoona et al. 2006b**
Providencia, *Bacillus* and *Alcaligenes* genera	Mung beans	Enhance the vigor and yield	**Akhtar and Ali, 2011**
ACCD producing bacteria	tomato	significantly increased shoot length, shoot fresh and dry mass, and the chlorophyll concentration	**Yan *et al.*, 2014**
Azotobacter and TRN1	Tomato	increased the root and shoot length, fresh and dry weight, number of leaves, chlorophyll a,b carotenoid contents, seedlings fresh and dry weight	**Hassan *et al.*, 2016**
Pseudomonas brassicacearum	Tomato	increased root elongation and root biomass	**Belimov *et al.*, 2007**

Accd producing bacteria	Chickpea	Increased the root length, shoot length, dry root weight, dry shoot weight, lateral root number, lateral root length and lateral root dry weight of chickpea	**Shahzad *et al.*, 2010**
ACCD producing bacteria	Pea	Significant increase in seedling length and root elongation	**Shaharoona *et al.*, 2006c**
Methylobacterium fujisawaense	Canola	Germination percentage was more in treated seeds and enhanced root elongation compared with control or mutant	**Madhaiyan *et al*., 2006**

Table 2.6. Alleviation of the impact of salinity stress on plant by PGPB containing ACC-deaminase Crops

PGPB	**Crops**	**Response**	**References**
P. fluorescens	Rice	Maintained root colonization potential by osmotolerance mechanisms	**Paul and Nair, 2008**

Bacillus, Microbacterium, Methylophaga, Agromyces, and *Paenibacillus*	Rice	Promoting the yield	**Bal *et al.*, 2013a**
Alcaligenes, Bacillus and *Ochrobactrum*	Rice	Positive impacts on germination percentage, shoot and root growth and chlorophyll content	**Bal *et al.*, 2013b**
Pseudomonas putida and *Pseudo- monas fluorescen*	Rice	Promote rice growth and colonize rice roots	**Etesami *et al.*, 2014**
P. putida, P. aeruginosa and *S. Proteamaculans*	Wheat	Increased plant height, root length, grain yield	**Zahir *et al*., 2009**
P. putida, Enterobacter cloacae, Serratia ficaria and *P. fluorescens*	Wheat	Improved growth and yield	**Nadeem *et al*., 2010**
Pseudomonas syringae, Pseudomonas fluorescens and *Rhizobium phaseoli*	Mung bean	Improving seedling growth and nodulation	**Ahmad *et al.*, 2011**
Azospirillum strains	Wheat	Increased shoot dry weight and grain yield	**Nia *et al.*, 2012**

Pseudomonas putida, Enterobacter cloacae, Serratia ficaria, and *Pseudomonas fluorescens*	Wheat	Enhanced germination percentage, germination rate, and index and improved the nutrient status	**Nadeem *et al.*, 2013**
Bacillus and *Hallobacillus*	Wheat	Enhance plant growth	**Ramadoss *et al.*, 2013**
Klebsiella sp.	Wheat	Increasing plant biomass and chlorophyll content	**Singh *et al.*, 2015**
B. subtillis and *Arthrobacter* sp	Wheat	Influence the growth and yield	**Upadhyay and Singh, 2015**
Bacillus licheniformis	Wheat	Increased of root and shoot length, fresh weight, and dry weight	**Singh and Jha, 2016**
ACC-deaminase PGPB	Maize	Increase in root-shoot length, fresh, dry weight, and chlorophyll pigments	**Nadeem *et al.*, 2006**
P. putida , P. fluorescens	Maize	Increase root-shoot length	**Kausar and Shahzad, 2006**

Pseudomonas syringae, Enterobacter aerogenes, and *Pseudomonas fluorescens*	Maize	Promoting the growth and yield	**Nadeem *et al.*, 2007**
P. syringae, P. bathycetes, E. aerogenes, *F. ferrugineum, P. fluorescenc*	Maize	Improved growth, yield and nutrition	**Nadeem *et al.*, 2009**
Pseudomonas syringae and *Pseudomonas fluorescens*	Maize	Significantly improved the yield of maize	**Zafar-ul-Hye *et al.*, 2014**
Rhizobium and *Pseudomonas*	Mung bean	Improve the growth, physiology and quality	**Ahmad *et al.*, 2013**
Brevibacterium epidermidis and *Bacillus aryabhattai*	Canola	Increased the seed germination	**Siddikee *et al.*, 2015**
Pseudomonas sp	Barley	enhanced root biomass	**Chang *et al.*, 2014**
Pseudomonas sp	oats	enhanced root biomass	**Chang *et al.*, 2014**

MATERIAL AND METHODS

The studies on "**Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylate Deaminase (ACCD) in improving growth and yield of cereal crops under salinity stress**" were carried out in the Department of Biological Sciences, SHUATS, Allahabad during the period 2013-2016. The materials and methods used during the studies are described in this chapter.

3.1. SHUATS Model Organic Farm [SMOF]: Collection site of soil samples

SHUATS Model Organic Farm (SMOF), Allahabad is located at 25o 24' 42" N latitude, 81o 50' 56" E longitude and 98 m altitude above the mean sea level. It has a sub-tropical and semi-arid climate with the monsoon commencing from July and withdrawing by the end of September. About 1000 mm of mean annual rainfall is unevenly distributed and most of it is received during the kharif. Apart from this, a few winter and summer showers are also received. Organic farming had been practiced for several decades on the campus. The SMOF covers an area of 2 hectares [5 acres or 8 bigha] and it was further developed during 2008 to 2017 under the National Project on Organic Farming (NPOF), which had a provision for certification. Lacon Quality Certification (P) Ltd. [Accreditation No. NPOP/NAB/006, Ministry of Commerce, Govt. of India] has been certifying SMOF during the past 7 years [Certificate No. ORG/SC/1009/001070] and the same is being continued currently.

3.2. Collection of soil sample

Soil samples (50g each), from different locations were collected from SHUATS Model Organic Farm (SMOF), Allahabad. The samples were collected from top 16 cm depth of sampling sites soil. The composite soil samples collected from a particular field in the brown paper bag labelled separately. Samples were brought to laboratory and stored at 4°C until used.

3.3. Isolation of bacteria

One gram of the soil sample was taken and added to 1ml of sterilized phosphate- buffered saline (Hi-media, pH 7.2) to make a dilution of 10^{-1}. Six-fold serial dilutions of each soil samples were prepared in sterilized distilled water and 0.5 ml of each dilution *viz.*, 10^{-4} to 10^{-6} was poured on their respective culture media. Nutrient agar medium (**appendix-A, 1.1**) for

total Heterotrophs, Kings Media for *Pseudomonas* spp. (**appendix-A, 1.4)** (**Ahmad *et al.*, 2008**), Yeast Extract Mannitol Agar **(appendix-A, 1.3**) (YEMA) for *Rhizobium* spp. (**Vicent,1970**), Macconkey agar (**appendix- A, 1.10.)** for coliform and Ashby's Agar (**appendix- A, 1.11**) for *Azotobacter* spp. (**Norris and chapman,1968**), contained in petri plate and spread uniformly by adopting spread plate method. The Petri plates were incubated at 28 ± 2°C for 24 h. Morphologically different colonies appearing on the plates were purified in the their respective culture media (HiMedia, India). The purified isolates were preserved at 4°C.

3.4. Characterization of bacteria for plant growth promoting (PGP) traits

Bacterial isolates were characterized for following PGP traits employing standard procedures.

3.4.1. Ammonia production (NH_3)

Bacterial isolates were screened for the production of ammonia in peptone water (**appendix-A, 1.8**). Freshly grown culture were inoculated in 10 ml peptone water in each tube and incubated for 48-72 h and after 2 -3 days Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was considered as a positive for ammonia production (**Cappucino and Sherman, 1992**). Qualitatively the isolates were designated with plus (+) sign from 1 to 3 depending on their efficiency to produce ammonia.

3.4.2. Hydrogen cyanide (HCN) production

HCN was detected according to the method of **(Bakker and Schippers, 1987)** King's medium (**appendix- A, 1.4**) was amended with 4.4 g glycine $l^{-1}$and bacteria were streaked on agar plate. A Whatman filter paper no. 1 soaked in 2% sodium carbonate and 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with parafilm and incubated at 37°C for 4 days. Development of yellow to red colour on filter paper indicated the positive HCN production.

3.4.3. Siderophore production (SD)

SD production was tested using Chrome Azurol S (CAS) agar plates (**appendix-A, 1.7**). Overnight culture was spot inoculated on CAS agar plate that was divided into equal sectors and incubated at 37^{o}C for 12 days. Appearance of orange halos around the colonies on the blue coloured agar indicated SD production (**Schwyn and Neilands, 1987**).

3.4.4. Indole acetic acid (IAA) production

IAA production was detected as described by **Brick *et al.*, (1991)**. Bacterial cultures were grown in peptone water (**appendix-A, 1.8**) at 37°C for 72 h. Fully-fledged cultures were centrifuged at 3000 rpm for 30 min. The supernatant (2 ml) was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski reagent (50 ml, 35% of perchloric acid, 1 ml 0.5 M $FeCl_3$ solution). Development of pink colour indicated IAA production.

3.4.5. ACCD activity

ACCD activity was performed as described by **Safronova *et al.*, (2006)**. The bacterial culture was grown in test tube containing 100 ml of liquid medium: KH_2PO_4 (2g), K_2HPO_4 (0.5g), $MgSO_4$ (0.2g), Glucose (0.2g). The medium was supplemented with 0.3g ACC or 0.19g $(NH_4)_2SO_4$ as a N source and incubated at 37°C for 24 -72hrs.The appearance of bacterial growth indicated the ACC deaminase activity of the bacteria.

3.4.6. Phosphate solubilization (PS)

PS of isolates were evaluated from the ability to solubilize inorganic phosphate. Pikovskaya's agar medium (**appendix-A, 1.5**) containing calcium phosphate as the inorganic form of phosphate was used in assay. A loopful of bacterial culture was streaked on the plates and kept for incubated at 28°C for 4-5 days. The appearance of transparent halo zone around the bacterial colony indicated the phosphate solubilizing activity of the bacteria (**Nautiyal, 1999**).

3.4.7. Chitinase production

Chitinase assay was performed as described by **Das *et al.*, (2010)**. Chitin plates were prepared with nutrient agar medium amended with 1 % (w/v) colloidal chitin. The plates were divided into equal sectors; spot inoculated with 10- 1l of overnight grown culture and incubated at 37° C for 96 h. Zone of clearance around bacterial colonies indicated chitinase production.

3.4.8. Catalase production

Bacterial cultures were grown in nutrient agar medium for 18-24h at 37^0C. The cultures were kept on clean slide with the help of loop and 2-3 drop mixed of H_2O_2 and observe the for gas bubbles. If the organism gave gas bubbles then its gives positive results (**Schaad, 1992**).

3.5. Characterization of potential PGPB for tolerance to abiotic stress

3.5. 1. Salinity

Salt tolerance of the organism was determined by inoculation in nutrient broth with different concentration of salt (05 to 20%) and incubated at 37^0C for 48-72 hrs. Growth in the medium was considered as tolerance to salt (**Damodaran *et al*., 2013**).

3.5. 2. pH

Tolerance to pH was determined by inoculated in nutrient broth with different pH (5 to 9) and incubated at 37^0C for 48-72 hrs. Growth in the medium was considered as tolerance to pH (**Damodaran *et al*., 2013**).

3.5. 3. Trace elements

The selected bacterial strains were tested for their tolerance to heavy metals by agar diffusion methods (**Cervantes *et al*., 1986**). Freshly prepared agar plates were amended with various soluble heavy metals namely Au, Hg, Ag, Cr, Cu, As, Ni, Zn, Al, Mo, Mn and Pb at various concentration ranging 0.6 to 3200 µg/ml and were inoculated with overnight grown cultures. Heavy metal tolerance was determined by the appearance of bacterial growth after incubating the plates at 37^oC for 24-48 hrs.

3.6. Susceptibility to Antibiotics

The bacterial strains were tested for their resistance to different antibiotics (viz., Cephalexin, Ampicillin, Cephataxime, Nalidixic, Neomycin, Streptomycin, Vancomycin, Kanamycin, Rifampicin, chloramphenicol, Trimethoprim, Tetracycline, Gentamycin) by agar diffusion method (**Bauer *et al.*, 1966**). The bacterial strains were inoculated in freshly prepared agar plates amended with specific antibiotic, incubated the plates at 37^0C for 48 hours and determined the antibiotic resistance by observing growth of the organisms.

3.7. Antioxidant enzyme activity

3.7. 1. Superoxide dismutase (SOD) activity

2.8 ml of EDTA buffer, 100 µl of Pyrogallol solution and 100 µl of bacterial homogenate were mixed to prepare the sample mixture and was assayed spectrophotometrically. Superoxide

dismutase activity was measured by the inhibition of pyrogallol autoxidation at 420 nm for 3 min according to the method of **Marklund and Marklund (1974)**. The enzyme activity was expressed as IU/mg protein, where 1U is the amount of enzyme required to bring about 50% inhibition of the autoxidation of pyrogallol.

3.7. 2. Catalase activity

2.8 ml of PB, 100 µl of bacterial homogenate and 100 µl of hydrogen peroxide were mixed together to prepare the sample mixtures that were assayed spectrophotometrically. The activity of CAT was determined by assaying the enzyme in the sample mixture according to the method described by **Beers and Sizer (1952)**. Change in optical absorbance was recorded spectrophotometrically at 240 nm for 3 minutes. One unit of enzyme activity was defined as micromoles of hydrogen peroxide decomposed per minute using molar extinction co efficient of hydrogen peroxide (43.6 per $Mole^{-1}cm^{-1}$).

3.7. 3. Measurement of Glutathione (GSH) activity through Ellman's Standard Curve

500 µl of bacterial homogenate was mixed with 500 µl of 5% TCA and was kept at 4˚C for 5 minutes, followed by centrifugation at 3000 rpm for 15 minutes at 4˚C in cooling centrifuge. The clear supernatant was taken for the preparation of sample mixture. 150 µl of this clear supernatant, 400 µl of DDW, 50 µl of NaOH, 300 µl of NaOH and 100 µl of DTNB stock solution were mixed to prepare the sample mixture. Absorbance change was recorded at 412 nm in these sample mixtures and according to the value obtained from the Ellman's Standard Curve; the amount of GSH in the sample mixture was calculated in µg/ml Protein. The amount of GSH in the bacterial homogenate was measured in µg/mg Protein according to the method of Ellman (1959). Here 50 µg/ml (0.002 gm) of reduced Glutathione (GSH) was prepared in 40 ml of PB. 10 test tubes were prepared having 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µlitres of GSH in each. Accordingly DDW, TCA, PB, NaOH and DTNB were added in each test tube. After 5 minutes of incubation at room temperature, absorbance was taken at fixed wavelength of 412 nm. GSH blank was also prepared in which GSH was omitted. Change of absorbance versus final concentration of GSH in the mixture was determined by constructed standard curve.

3.8. Plasmid curing

Each strain was checked for their resistance towards a particular antibiotic and metal. For metals, the highest concentration which showed growth of a particular strain was taken for plasmid curing. For plasmid curing, 200µg/ml of acridine orange solution (mutagenic agent) was prepared in distilled water. 9 ml of peptone water and 10 ml of acridine orange solution were autoclaved separately. 9 ml of peptone water was mixed with 1 ml acridine orange solution. In another tube, 5 ml of peptone water was inoculated with bacterial culture at 37^0C in the morning. After about 7-8 hrs. 0.1 ml of this broth was transferred to a fresh 10 ml solution of acridine orange and incubated at 37^0C for 24 hrs under shaking conditions. Appropriate dilutions were placed on NA plate to obtain about 100 colonies. These colonies were picked aseptically and transferred on Antibiotic and metal infused plates of nutrient separately towards which the organism showed resistance earlier. The colonies which grow on the plate were those which still showed resistance and those which were cured, failed to grow on the plate since they were plasmid borne genes and were sensitive to the particular metal or antibiotic. (**Ramteke *et al*., 2012**).

3.9 Morphological, Biochemical and Molecular identification of potential PGPB

3.9.1. Morphological and biochemical characterization of potential PGPB

The isolates based on morphological observation and biochemical characterization were identified. The tests involved, were Gram staining, catalase, citrate utilization, indole test, Vogus Proskaur test, methyl red test, citrate, H_2S production, sugar fermentation etc. (**Aneja, 2001**).

3.9.2. Bacterial Growth Conditions and DNA Extraction

The loopful culture of 1-day-old cultures was transferred to 100 mL of their respective culture media, usually NB (**Appendix-A, 1.1**) medium and incubated at 28±2°C for 24 h. The fully grown cultural broth was settled through centrifuged at 10000 rpm for 10 min, washed the pellets twice with sterile distilled water, discard the supernatant. The genomic DNA of all bacterial isolates was extracted from collected pellete using the TE buffer method (**Appendix-B, 2.5**) (**Kumar *et al*., 2013**). The quality and concentration of the genomic DNA was assessed using a spectrophotometer (Shimazdu UV-160), which measured the UV absorbance at 260 and 280 nm and computed the 260/280 absorbance ratio. DNA resuspended in 50µl of TE

buffer (**Appendix-B, 2.5**) and concentration of the genomic DNA was quantified by use of ethidium bromide fluorescence showed in **Fig. 3.1**.

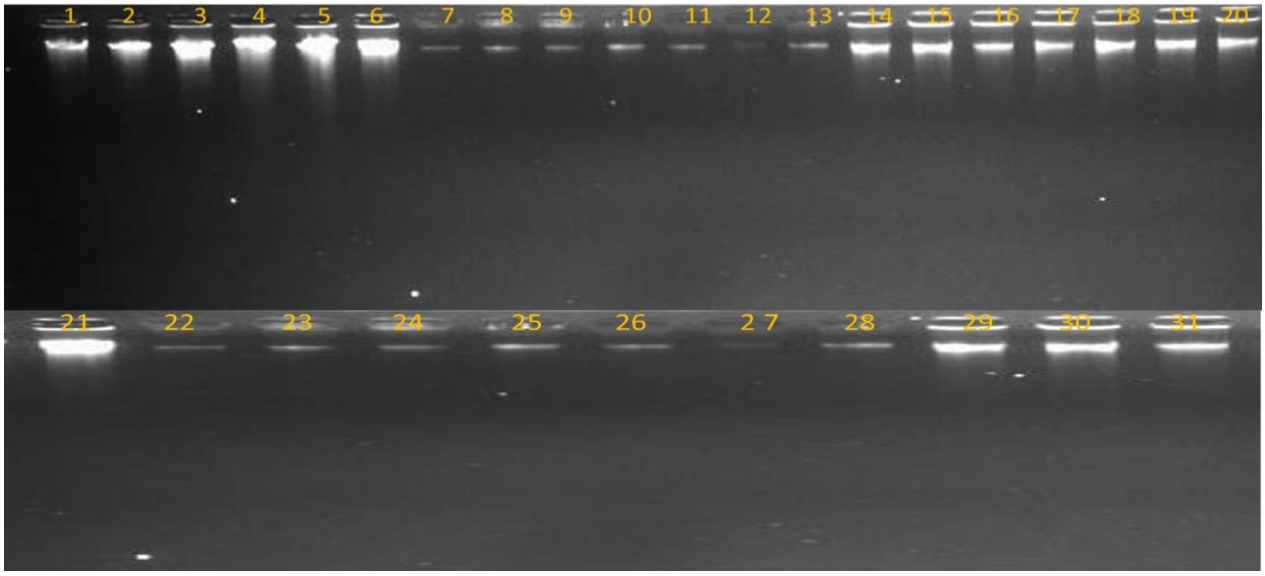


Figure 3.1. Genomic DNA extraction of different Bacterial isolates by TE buffer method, representing in lanes 1–31 were mentioned in table

3.9.2. Molecular identification and PCR amplification

The universal primer pair 16S rRNA was used for amplifying and sequencing the bacterial rDNA 16S region (**Woo *et al.*, 2006**). The nucleotide sequences were 16SF (5'-AGAGTTTGATCCTGGCTCAG-3') and 16SR (5'-AAGGAGGTGATCCAGCCGCA-3') and (Bangalore Genei, India). Amplification reactions were prepared in a total volume of 50µL containing 4µL of 5× Gitschier buffer, 2.5µL of dNTP mixture (2.5 mM each), 2 units of Taq DNA polymerase (5 U/µL) (Bangalore Genie, India), 50 pmol each of forward and reverse primers and 2.5µL of 50 ng DNA template. Thermal cycling (Bio-rad) consisted of a 2 min initial denaturation at 95°C, followed by 40 cycles of elongation (denaturation at 94°C for 1 min, annealing at 55°C, 58°C and 58°C, respectively (for each gene mentioned above), for 1 min, and extension at 72°C for 1 min), and ending with a final extension at 72°C for 10 min. A 100-bp ladder (MBI Fermentas) was used as a molecular size standard marker. The PCR products were separated by electrophoresis (at 75 V cm^{-1} for 50 min) on 1.5 % (w/v) agarose gels (**Appendix-B, 2.7**) with 1×TAE buffer (**Appendix-B, 2.6**). The gels were then stained

with ethidiumbromide to visualize products under UV light using a gel documentation system (Bio-Rad, Philadelphia, PA, USA).

3.9.3. Phylogenetic analysis of isolated microorganism

For species identification, 16S F and 16S RNA gene sequences were submitted to the BLAST interface in NCBI (http://blast.ncbi.nlm.nih.gov). All positions containing gaps and missing data were eliminated from the dataset. Phylogenetic analyses were performed in MEGA 4.0 (**Tamura et al., 2007**). 16S F and 16S RNA gene sequences were analyzed using the maximum parsimony (**Eck and Dayhoff, 1966**) approach of close-neighbor-interchange algorithm with search level 3 (**Nei and Kumar, 2000**), in which the initial trees were obtained with the random addition of sequences (10,000 replicates). In all cases, to infer the consensus, phylogenetic trees bootstrapping with 10,000 data replicates was conducted (**Felsenstein, 1985**). The nucleotide sequences of 16S F and 16S RNA gene were deposited in NCBI GenBank.

3.10. Laboratory studies on inoculation effect of PGPB on Seed germination and growth parameters of cereal crops

3.10.1. Details of cereal crops

The investigation was carried out with different cereals crops obtained from Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture Technology and Sciences, Allahabad. They are listed below:

3.10.1.1. Rice

S. No.	Varieties	Remarks
1.	Sahbhagi	Drought- tolerant, released and notified in 2010 in India and Nepal as ‘Sukha Dhan 3’ and in Bangladesh as ‘BRRI Dhan 56.
2.	Ginphou	Manipur salt tolerant
3.	Leimaphou	Manipur salt tolerant
4.	RC 4	Manipur salt tolerant

5.	RC 5	Manipur salt tolerant
6.	RC 6	Manipur salt tolerant
7.	RCM-10	Manipur salt tolerant
8.	CAUR-1	Manipur salt tolerant
9.	CAUR-3	Manipur salt tolerant

3.10.1.2. Maize

S. No.	Verities	Remarks
1.	Kanchan	GBPUAT,Pantnager ,1982, Kharif
2.	Azad Uttam	C.S. Azad university, Kanpur, 1991, , Kharif
3.	Saradmani	C.S. Azad university, Kanpur,2008, Rabi
4.	Navjyot	PAU,Ludhiana,1983, , Kharif
5.	SHUATS MS -2	State varietal release subcommittee, U.P.

3.10.1.3. Millets

S. No.	Millets	Remarks
1.	Normal sorghum ,SCV 20	South variety, Kharif
2.	Sweet sorghum,CSH 16	Maharashtra, 1962, Kharif
3.	Finger Millets CO 14	South variety, Kharif
4.	Pearl Millets MH 1642	South variety, Kharif

3.10.1.4. Wheat

S. No.	Varieties
1.	NIDW295XHI8636
2.	HPD153XMASA499
3.	SHUATS DW3
4.	SHUATS DW5
5.	RAJ1535
6.	SHUATS DW6

7.	SHUATS DW2
8.	SHUATS DW1
9.	AVKD-3XRD1008
10.	DBPY-02-03XMASA499
11.	HI8653
12.	DBP-01-11
13.	RAJ6560
14.	HD2009
15.	NIDW-295
16.	PDW-300
17.	HPD-153 XMASA499
18.	DBPY-02-03X RD1008
19.	3'AKDW-2997
20.	DBPY-02-03XMASA 499(White Awn)
21.	HPD153X HASA499 (White Awn)
22.	NIDW-299 XHI8636
23.	HPD153X RD1088
24.	AVKO-02 X HI8636 (White Awn)
25.	AAI-W6
26.	K-9162
27.	Raj 3077
28.	HD-2786

3.10.2. Inoculation effect of STPGPB on seed germination and other growth parameters of cereal crops

Sterilized culture test tubes were filled with 9ml of distilled water and inoculated with STPGPB. STPGPB were inoculated with the help of an inoculating needle. Cereal seeds treated with the STPGPB. One test tube was left un-inoculated and treated as control. Twenty five seeds were counted and dropped into the test tubes. The test tubes were left standing for 30 minutes. The seeds were then transferred to petri plates lined with germination paper. The Petri-plates were incubated at 25 ^{o}C for up to 09 days **(Nandakumar *et al*., 2001)**.

3.10.2.1. Inoculation effect of STPGPB on seed germination and growth parameters of cereal crops under salt stress

Above processes (3.10.2) as followed with salt stress**.** Inoculated seeds were then subjected to germination on germination papers and irrigation by salt water (1-2%) with alternate days. After 3 days the seedling were taken out for seed germination and root and shoot length and the data were recorded.

3.10.2.2. Inoculation effect of STPGPB on seed germination and growth parameters of cereal crops in presence of ammonium sulfate (substitute of ACC) under salt stress

Above processes (3.10.2) as followed and irrigation with ammonium sulfate (substitute as ACC) and different concentration of salt water with alternate days. Seeds were germinated 9days at 25°C. After 3 days the seedling were taken out for seed germination and root and shoot length and the data were recorded.

3.10.2.3. Observations Recorded

3.10.2.3.1. Inoculation effect of STPGPB on seed germination

The germination percentage was calculated according to the prescribed standards given by (**ISTA, 1999)**.

Seed Germination % = (No. of germinated seed / No. of total seeds) X 100

3.10.2.3.2. Root and Shoot length

The root and shoot readings were taken on the 3^{rd}, 5^{th}, 7^{th} and 9^{th} DAI (days after inoculation). The root and shoot readings were measured with the help of a scale in centimetres. The genotypes that showed the best response to the inoculation were identified.

3.11. Inoculation effect of PGPB on morphological and yield parameters of cereal crops under field condition

Inoculation effect of STPGPB on morphological and yield parameters cereal crops viz. finger millet, wheat and maize under field condition by standard protocols they are followed:

3.11.1. Plant height (cm)

The plant height (cm) was recorded from the ground level to the growing tip of the main shoot. Measurement was taken at 90 DAS from five tagged plants randomly in each treatment of different sets, and the average height was calculated and expressed in centimetre.

3.11.2. Number of tiller per plant

The data on number of tiller per plant was recorded at 100 days from five tagged plants randomly in each treatment of different sets.

3.11.3. Spike length (cm)

The average spike length (cm) of five plants on the main culm from the base of the spike to the top of the last spikelet excluding awns recorded in centimetre.

3.11.4. Spike weight (g)

The average spike weight (g) of five plants was taken in gram.

3.11.5. Number of grains per spike

The number of grains per spike was recorded by counting the number of grains in one spike of respective wheat plant.

3.11.6. Grain yield per plant (g)

Grain yield per plant (g) was recorded from yield of grain per plant each obtained after harvesting and threshing.

3.11.7. Flag leaf width

The flag leaf width was measured in centimetre in middle part of the leaf from one margin in each of the randomly selected flag leaf.

3.11.8. Length flag leaf

The length flag leaf was measured in centimetre from the collar junction of the blade and leaf sheath to the tape of blade.

3.11.9. Test weight (g)

The Test weight (g) was recorded by weighing 1000 grains in each entry.

3.11.10. Fresh weight of a plant

The Fresh weight of a plant taken after harvesting of a crop, in each treatment of different sets and the average was worked out.

3.11.11. Dry weight of the plant (g)

Dry weight of the plant (g) was determined by autoclaving three different tagged plants in each treatment of different sets for 24 hours at 60^{o}C and average was worked out.

3.11.12. Days to 50% flowering

Count the no of days to 50% flowering at each treatment of different sets and average was worked out.

3.11.13. Number of finger per ear

The number of finger from nine tagged plant's ear was counted in each treatment of different sets and the average was work out.

3.11.14. Finger length (cm)

The average finger length from nine plants ear on the main calm from the base of the finger to the top was recorded in centimetre.

3.11.15. Root length (cm)

The average root length from nine tagged plants were recorded in centimetre.

3.11.16. Total chlorophyll

Total chlorophyll content was determined according to the method of **Arnon (1949).** Fresh leaves were collected and sample (200 mg) was taken from fully mature leaf. The sample was mixed with 2 ml (80%) acetone and ground well. These mixtures were centrifuged at 10,000 rpm for 5 min. After centrifugation, the supernatant was removed and transferred into a fresh

test tube. Acetone was added to test tube containing the sample and reached the volume up to 6 ml. Absorbance of the samples was read at 645nm and 663nm using spectrophotometer. The total chlorophyll content was calculated by using the following formula, Total chlorophyll (µg/ml) = 20.2 (A_{645}) + 8.02 (A_{663}).

3.11.17. Relative water content (%)

The relative water content, RWC (%) was estimated by the method of **Barrs and Weatherly (1962)**. Five leaf discs were collected and weighed by balance. This was considered as fresh weight. The weighed leaf discs were allowed to float on distilled water in a petridish and allowed to absorb water for four hours. After four hours, the leaf discs were taken out and their surface was blotted gently and weighed. This was referred to as turgid weight. After drying in hot air oven at 72^0C for 48 hours, the dry weight was recorded and RWC was calculated by applying the following formula, RWC (%) = Fresh weight – Dry weight/ Turgid weight – Dry weight x 100.

3.11.18. Protein estimation

Protein estimation was performed by adopting the method given by **Lowry *et al*. (1951).** The protein content was determined by the standard curve prepared out of the Bovine serum albumin protein and absorbance was measured at 660 nm (**appendix- C, 3.1**).

3.11.19. Total carbohydrate content (g)

The total carbohydrate content (g) in plants were determined according to the methods of **Hedge and Hofreiter (1962)**. Fully mature leaves were collected from plants and dried. Sample (200 mg) was taken and added 10 ml water into test tube and boiled for 1 h in a water bath. One ml of extract was taken from the test tubes and 3ml of 3% Anthrone reagent (Sigma Aldrich) was added to the extract and the mixtures were kept in waterbath for 30 min at 100^0C. Samples (2 ml) were taken in cuvettes and read the absorbance at 630 nm using glucose as a standard and the amount of carbohydrate present in the sample tube was calculated (**appendix- C, 3.2**).

3.11.20. Harvast index (%)

The Harvest index **(%)** was calculated by applying the following formula, HI = Seed yield per plant (g)/ Total dry weight per plant (g) x 100.

3.12. Statistical Analysis

The experiments were analysed using **Student's *t*-test**. The *p*-value is used in the context of null hypothesis testing in order to quantify the idea of statistical significance of evidence. An informal interpretation of a *p*-value, based on a significance level of about 10%, might be:

1. P<0.05 low value
2. P<0.01 Moderate value
3. P<0.001 High value

RESULTS AND DISCUSSION

The results of detailed studies on **"Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane-1-carboxylate Deaminase (ACCD) in improving growth and yield of cereal crops under salinity stress" are presented in this chapter.**

The results comprising 650 PGPB isolated from SHIATS Model Organic Farm (SMOF), Allahabad, their cultural, morphological, biochemical and molecular characterization; determination of their PGP traits and their role in enhancement of seed germination and growth parameters of various cereal crops under normal and salt stress are presented here.

4.1. Chemical characterization of soil from SMOF

The chemical characterization of soil from SMOF is based on various parameter like pH, electrical Conductivity(EC), total organic carbon, available Nitrogen (N), available Phosphorus (P_2O_5) and available Potassium (K_2O). Chemical properties of soil from SMOF in different seasons are showed varying values of parameters, given in **Table 4.1**. In general higher values of all chemical properties determined were noted in rainy season as compared to winter and summer seasons. Exceptionally high content of available potassium (K_2O 302-320 kg ha-1) noted in all the seasons. However, values for all other chemical parameters were comparatively low. Few other workers have reported similar trend on values for chemical properties of soil from organic farm (**Derrick and Dumeresq, 1999; Gosling and Shepherd, 2005; Domagała-Świątkiewicz and Gąstoł, 2013**). This study leads us to conclude the nutrient's quanity of organic farm soil for combat the problems related to soil nutrients and addition of amount of fertilizers to soil to make it productive. Soil pH, electrical conductivity (EC), organic matter (OM), available phosphorus (AP), and potassium (AK) are some of the most important indicators of soil fertility. These soil parameters are highly variable in space and time, especially in agricultural areas, with implications for crop production.

Table 4.1. Chemical properties of Soil from SMOF

Parameters	Season		
	Winter	Summer	Rainy
pH	7.8	7.8	7.7
Available OC (%)	0.22	0.36	0.44
Available N (%)	0.02	0.03	0.04
Available P_2O_5 (kg ha^{-1})	9.00	18.00	22.01
Available K_2O (kg ha^{-1})	302.00	313.00	320.11
Available OS (ppm)	10.66	13.07	16.85
Available Zn (ppm)	0.52	0.88	1.30
Available Fe (ppm)	8.26	12.16	15.36
Available Mn (ppm)	10.26	15.00	17.50
Available Cu (ppm)	0.82	1.00	1.14

4.2. Bacterial diversity of soil from SMOF

Bacterial diversity and their seasonal abundance is given **table 4.2**. The microbial populations were significant increase during initial stages of composting process for all the three seasons. The heterotrophs, coliforms, *Pseudomonas* spp., *Azotobacter* spp., *Rhizobium* spp., were dominating species SMOF. From the present investigation, it can be concluded that, the summer season microbial activities faster because the favourable environmental conditions for supporting the proper organic content degradation, therefore, summer season for obtained better quality of compost than rainy season.

Diversity in soil microbial populations in organic farm is pivotal for nutrient uptake and disease suppression. Bacterial diversity of SMOF comprised heterotrophs, coliforms, *Pseudomonas* spp., *Azotobacter* spp., *Rhizobium* spp. as summarized with total viable Count CFU $x10^5$ g^{-1} (**table 4. 2**). Overall bacterial counts in soil from organic farm were significantly ($P<0.01$) higher in summer as compared to winter and rainy seasons and diversity of heterotrophs and *Rhizobium* sp. were relatively higher in CFU compare to other bacterial groups.

We observed rich bacterial diversity in soil from SMOF both in terms of their types and functional (PGP) traits (**Table 4.2 and Figure4.1**). Organic farming potentially offers a means of recovering functional evenness in an ecosystem. A number of studies have confirmed that soil microbes are often more diverse and abundant under organic than conventional systems in various soils. The abundance of some soil microbial groups was increased under an organic system. As observed by us, other researchers have reported increased soil bacterial biomass, activity and bacterial functional and taxonomic richness and diversity of organic farm (**Gardner *et al.*, 2011; Lopes *et al.*, 2011; *Das and Dkhar*, 2011 ; Grantina *et al.*, 2011; Schmid *et al.*, 2011; Wang *et al.*, 2012; Stockdale *et al.*, 2013; Hartmann *et al.*, 2014; Lupatini *et al.*, 2017**). They have also reported higher population of microorganisms in organic farm as compared to conventional farms. Positive effect of organic farming on biodiversity and increase in species richness has been also recently revealed by meta-analysis (**Rahmann, 2011; Tuck *et al.*, 2014**). Soil biodiversity is important for soil resistance and resilience (**Girvan *et al.*, 2005; Brussaard *et al.*, 2007**). In the present investigation rich seasonal bacterial diversity and biomass representing several bacterial groups such as heterotrophs, coliforms, *Pseudomonas*, *Azotobacter* and *Rhizobium* in SMOF was observed (**Table 4.2**).

Table 4. 2. Seasonal abundance of organisms in soils from SMOF

Organisms	**Total Viable Count CFU x10^5 g^{-1}**		
	Winter	**Summer**	**Rainy Season**
Heterotrophs	4.0	8.4**	3.5
Coliforms	3.4*	6.0**	1.7
Pseudomonas spp.	3.6**	8.2***	1.4
Azotobacter spp.	3.3	7.2**	2.5
Rhizobium spp.	4.0	7.6*	2.8

CFU=Colony forming unit,*p<0.05; **P<0.01; ***p<0.001

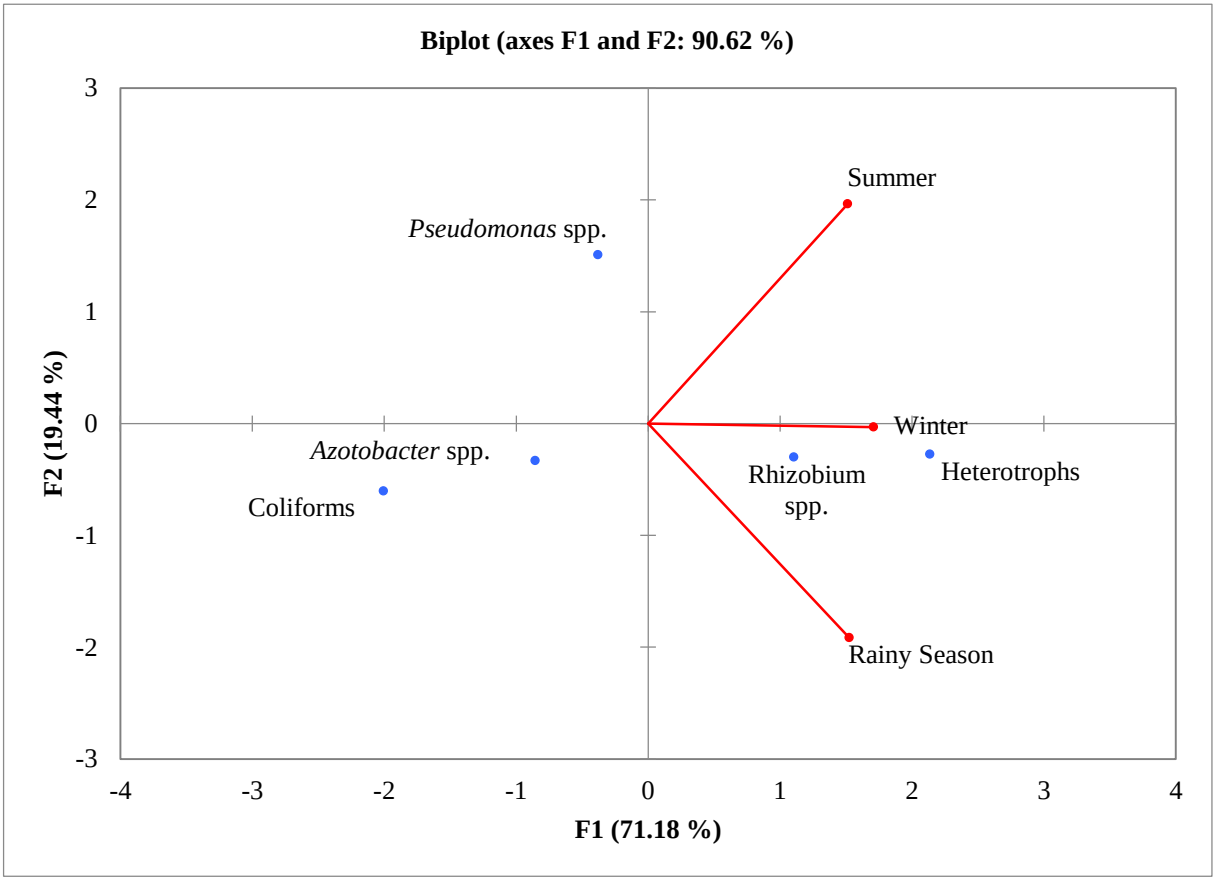


Figure 4.1 PCA biplot of different groups of bacteria at three different season under organic farming

A relationship between bacterial diversity and different seasons were determined as illustrated by a bi-plot (**Fig.4.1**). The first two principal components axis of the bi-plot accounted for 71.1 % (F1) and 19.4% (F2) of the total variation of the bacterial diversity and different seasons. In this bi-plot, bacterial diversity was located very far from the origin of biplot, indicating strong bacterial diversity in different seasons. Eigen values of the first and second components were 2.135 and 0.583, respectively.

4.3 Characterization of PGP traits among bacteria for soil from SMOF

Six hundred and fifty bacterial isolates from SMOF were screened for PGP traits and majority of them produced multiple PGP traits (**Table 4.3**).

Table 4.3 Characterization of bacteria for PGP traits in soil bacteria from SMOF

Organism	**No.**	**Incidence of PGP Traits (%)**						
		NH3	**HCN**	**SD**	**IAA**	**ACCD**	**PS**	**Catalase**
Heterotrophs	149	145(97.3)***	41(27.5)	128(85.9)***	135(90.6)***	106(27.5)**	29(19.4)	113(75.8)**
Coliforms	107	86(80.3)***	3(2.8)	35(32.7)**	80(74.7)***	78(72.8)***	43(40.1)**	86(80.3)***
Pseudomonas spp.	132	121(91.6)***	81(61.3)**	111(84.0)**	122(92.4)***	118(89.3)**	72(54.5)*	123(93.1)***
Rhizobium spp.	132	129(97.7)***	27(20.4)	76(57.6)**	121(91.6)***	112(84.8)***	65(49.2)**	117(88.6)***
Azotobacter spp.	130	125(96.1)***	28(21.5)	99(76.1)**	125(96.1)***	97(74.6)**	34(26.1)	112(86.1)***
Total (%)	650	606(93.2)***	180(27.6)	449(69.0)**	583(89.6)***	511(78.6)***	243(38.0)*	551(85.0)***

***p<0.05; **P<0.01; ***p<0.001, Production of ammonia= NH_3, hydrogen cyanide= HCN, Siderophore =SD, indole acetic acid = IAA, 1-aminocyclopropane- 1-carboxylate deaminase =ACCD and phosphorus solubilization activity = PS**

Several important bacterial characteristics, such as biological nitrogen fixation, phosphate solubilization, ACC deaminase activity, and production of siderophores and phytohormones, can be assessed as PGP traits. Isolates were predominately positive to production of ammonia (NH3) (93.2%), siderophore (SD) (69.0%), indole acetic acid (IAA) (89.6), 1-aminocyclopropane- 1-carboxylate deaminase (ACCD) (78.6%). Activity of phosphorus solubilization (PS) and production of hydrogen cyanide (HCN) were detected in 38.0 and 27.6% isolates, respectively. Production of all PGP traits detected was higher among *Pseudomonas* spp. as compare to other bacterial groups isolated from organic farm (**Table 4.3**). Production of HCN by coliforms was lowest among all other groups of bacteria.

Production of ammonia was observed predominantly among isolates from SMOF. In an earlier study production of ammonia was detected in 74% bacterial isolates from rhizospheric soil of chickpea of conventional farm in the vicinity of Allahabad (**Joseph *et al.*, 2007**) whereas the present study showed higher ammonia producers (93%) from the organic farm of the same vicinity. In addition to the source of nitrogen in soil its involvement in antagonistic interactions with soil pathogens that result in disease control is reported (**Saraf *et al.*, 2008**). **Narula and Gupta (1986)** found that inoculation of wheat and barley with ammonia excreting strains caused increased dry weight and enzyme activity.

Catalase is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). It is well known that the products of oxygen reduction such as hydrogen peroxide can be highly toxic for cells but catalase can split hydrogen peroxide into molecular oxygen and water and thus prevent cells from damage by reactive oxygen species (**Yao *et al.*, 2006**).

Production of IAA is widespread among rhizobacteria (**Patten and Glick, 1996; Khalid *et al.*, 2004; Spaepen *et al.*, 2007**) and is one of the most physiologically active auxin or phytohormones implicated in the regulation of plant growth and development. Researchers have observed an increase in root hairs and lateral roots thus increasing the total root surface leading to an enhanced mineral uptake from the soil by inoculation of plants with PGPB with ability to produce high levels of IAA (**Patten and Glick, 2002; Aloni *et al.*, 2006; Fukaki *et al.*, 2007**). PGPB mediated induction of seed germination and root and shoot length was demonstrated by several researchers (**Anitha and Das, 2011; Ramteke *et al.*, 2012**).

In addition to its effect on growth of plants, microbially produced IAA has been suggested to trigger an increased level of protection against external adverse conditions by coordinately

enhancing different cellular defense systems. Because of this multiple effects on plants, many pathways such as tryptophol, tryptamine, indole-3-pyruvic acid and indole-3-acetamide pathways have been reported to be evolved in microorganism for IAA production (**Gravel *et al.*, 2007**). Thus, IAA production-based screening can be considered as an effective tool for detecting beneficial microorganisms with regulatory effect on plant growth (**Ali and Hasnain, 2007; Govindarajan *et al.*, 2007**).

Microbes play an important role in the acquisition and transfer of nutrients in soil (**Richardson, 2001**). Therefore, the utilization of soil microbes to activate minerals and enhance nutrient uptake in plants has attracted increasing attention in sustainable agriculture (**Fayez and Mahmoud, 2006**). Phosphorus (P) is one of the major essential macronutrients for plants. In India, it is estimated that there are almost 260 million tons of phosphate rock deposits and this material should provide a cheap source of phosphate fertilizer for crop production (**FAI, 2002**). Although in soil P is available abundantly its bioavailability in soil remains low due to the chemical transformations of P into insoluble forms (**Rodriguez and Fraga, 1999**) and thus a major constraint to the plant growth and crop production (**Chiquito-Contreras *et al.*, 2012**). Phosphate-solubilizing bacteria (PSB) have been considered as one of the possible alternatives for mediating inorganic phosphate solubilization and increasing its availability to the plants (**Rodríguez *et al.*, 2006**).

In the present study the observed significant number of organisms particularly 38% that displayed phosphate solubilizing activity (**table 1**). A study by **Kaur and Reddy (2014)** suggested that PSBs play an important role in improving crop productivity in organic farming. They noted significant increase in the biometric parameters (shoot height, shoot and root dry biomass) of maize and wheat plants after treatment with PSBs. Similar results were noted by other investigators (**Dugar *et al.*, 2013; Hassimi *et al.*, 2013**; **Ranjan *et al.*, 2013**). In addition to PS activity, PSBs may also improve the plant productivity by producing other secondary metabolites. There are several evidences related to plant growth promotion by PSBs through the production of indole acetic acid (IAA) and siderophores (**Hariprasad and Niranjana, 2009**). Few earlier studies established the relationship between P-Solubilizing Index (PSI) and growth and physiological parameters of Phosphate solubilizing endophytes (**Parihar *et al.*, 2003; Parihar and Ramteke, 2003**).

Iron (Fe) is an essential plant micronutrient and microbial siderophores enhance Fe uptake by plants (**Kloepper *et al.*, 1980; Katiyar and Goel 2004; Dimkpa *et al.*, 2009**) and thus plays

an important role in plant growth promotion. Although large portion of Fe is present in soil it acts as a limiting factor for plant growth because its existence in the form of highly insoluble ferric hydroxide. Bacteria secrete siderophores to solubilize Fe from their surrounding environments by forming a complex ferric-siderophore and provide it to the plants for growth promotion (**Andrews *et al.*, 2003**). Siderophore mediated suppression of phytopathogens was reported by (**Jasim *et al.*, 2015)**. Here, a physiological response is implied by the fact that HCN production is induced by iron (**Bakker and Schippers, 1987; Keel *et al.*, 1989; Voisard *et al.*, 1989)** and that it is under strong influence of quorum sensing (**Pessi and Haas, 2000).** The latter is likely to happen particularly in the rhizosphere, where root exudation promotes high bacterial counts.

The enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD), widely spread in bacteria (**Belimov *et al.*, 2001, Ghosh *et al.*, 2003; Ma *et al.*, 2003; Dey *et al.*, 2004; Glick, 2005; Hontzeas *et al.*, 2005; Blaha *et al.*, 2006; Madhaiyan *et al.*, 2006; Duan *et al.*, 2009**), cleaves ACC, the immediate precursor of ethylene in plants and convert into ammonia and α-ketobutyrate (**Glick *et al.*, 1998)**. These products of ACC cleavage are potential nitrogen and carbon sources (**Glick *et al.*, 1998**) that can play a role in the microorganism's fitness under stressful situations. Under stress conditions plants produce higher levels of the phytohormones ethylene, which means that the plants also produce higher levels of ACC (**Glick *et al.*, 2007**).

Under stress conditions, not only plant produce increased amount of ACC, the vast majority of rhizosphere microorganisms produce the phytohormone indole acetic acid (IAA) which acts to loosen plant cell walls thereby facilitating root exudation. Bacterial IAA production has also been shown to increase ACC synthase expression in plants (**Kende, 1993**). Thus, microorganisms that can both produce IAA and utilize ACC may have a competitive advantage over other soil microorganisms (**Glick *et al.*, 1998, Stearns *et al.*, 2012**). It is known that ACCD modulates ACC metabolism and not only is related to plant growth promotion abilities, but may also play multiple roles in microorganism's developmental processes (**Nascimento *et al.*, 2014**) and protect the plant from stress (**Subramanian *et al.*, 2015**).

A study reveals the role of ACCD in influencing the senescence of flowers and thereby increasing the shelf life (**Ali *et al.*, 2012; Jasim *et al.*, 2015**). Additionally, recent results suggesting a considerable degree of horizontal ACCD gene transfer events (**Nascimento *et al.*, 2014**). Hence, exploration of more ACCD gene from diverse bacterial sources and in depth understanding of functioning of this enzyme especially in plant physiology signifies its

importance. This may be the key in a variety of important agricultural and biotechnological applications of ACCD genes and their expression (**Nascimento *et al.*, 2014**).

Volatile compound HCN produced by rhizobacteria is mainly considered as biocontrol agent against phytopathogenic fungi (**Ramette *et al.*, 2003; Ahmad *et al.*, 2008; Rezzonico *et al.*, 2007; Siddiqui *et al.*, 2006**).

It is proposed that HCN interrupts functioning of many enzymes (**Cooper and Brown, 2008**) or protein carriers and as a result inhibits the growth of certain organisms. **Wei and co-workers(1991)** expressed the possibility that HCN induces systemic resistance in some plants making them resistant to phytopathogen attack, however, proposed regulatory functions of HCN in plants need further work (**Siegie´n and Bogatek,2006**).

Contradictory to the role of HCN as biocontrol agent, several researchers concluded that HCN is hardly a universal biocontrol agent and even caused phytotoxic effects in most in vitro experiments (**Alström and Burns, 1989; Pal *et al.*, 2000; Kremer and Souissi, 2001; Rudrappa *et al.*, 2008; Blom *et al.*, 2011**). In a recent study by **Rijavec and Lapanje (2016)** have clearly shown that there is no correlation between the amount of HCN produced by a particular strain and its ability to inhibit the growth of phytopathogenic bacteria or fungi. On the other hand they proposed a new role for HCN production by rhizospheric bacteria that is HCN increases phosphate availability for rhizobacteria and plant hosts especially in oligotrophic alpine environments. They further stated that the understanding of the involvement of HCN producing bacteria in geochemical processes will be of great importance in the future.

4.3.1. Seasonal variations of PGP traits among soil bacterial from of SMOF

This study investigates the prevailing seasonal changes of PGP traits and microbial community for mesophilic bacteria at different degradations stages of organic farm soil. Seasonal variations of PGP traits among all bacterial isolates from soil of SMOF are shown in **Fig. 4.2.** In all the three seasons, majority (>60%) of heterotrophs were positive to production of ammonia, IAA, ACCD and catalase (Fig.4. 2A). However, in summer none of them showed siderophore activity and few of them (<5%) were positive to PS activity. In summer none of the heterotrophs were positive for production of HCN. As compared to rainy season significant number of heterotrophs were positive to siderophore ($p<0.001$) and PS activity ($p<0.01$) in winter season.

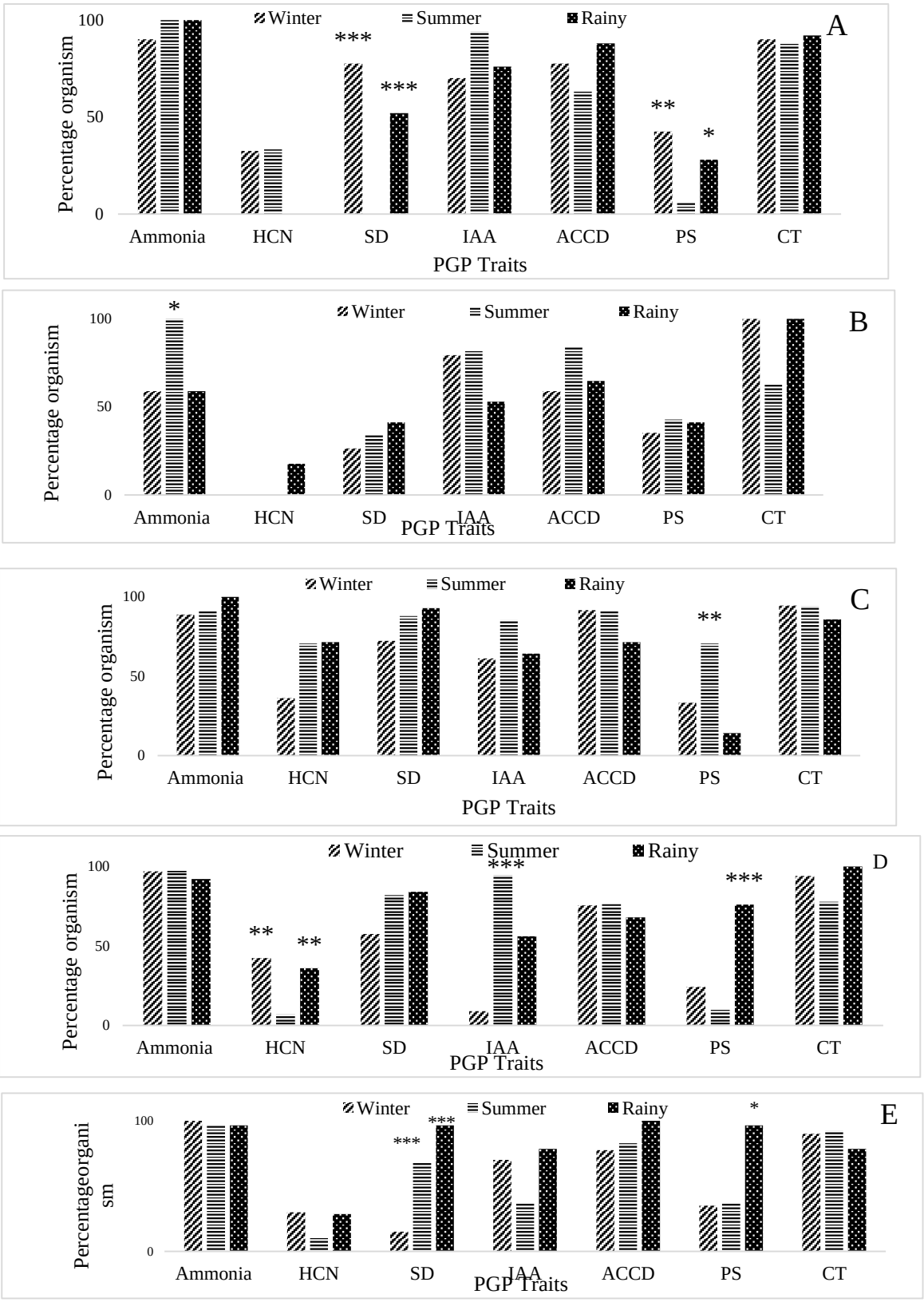


Figure 4.2. Seasonal variation of PGP Traits in bacterial isolates SMOF

***p<0.05; **P<0.01; ***p<0.001, A= Heterotrophs, B= Coliform, C= *Pseudomonas* spp., D= *Rhizobium* spp., E= *Azotobacter* spp., production of ammonia =NH3, hydrogen cyanide =HCN, siderophore = SD, indole acetic acid = IAA, 1-aminocyclopropane- 1-carboxylate deaminase =ACCD, phosphorus solubilization activity = PS**

All the coliforms found positive for catalase both in winter and rainy seasons and ammonia in summer season (**Fig. 4.2 B**). Both in winter and summer, around 80% of them showed production of IAA. ACCD activities in majority of coliforms were noted in summer season. Majority (>60%) of *Pseudomonas* spp. displayed all the PGP traits in all the three seasons except production of HCN and PS activity in winter (**Fig. 4.2C**).

Production of ammonia and catalase was >95% isolates of *Rhizobium* spp. at all the three seasons (Fig.4. 2D). As compared to winter and summer, in rainy seasons production of ACCD, siderophore and PS activity was detected in significantly high number ($p<0.001$) of isolates of *Rhizobium* spp.

As the seasonal variations are concerned for the PGP traits among *Azotobacter* spp., production of HCN was noted lowest in all three seasons as compared to other PGP traits (Fig. 4.2E). Production of IAA in summer and PS activity in rainy season was in significantly high ($p<0.001$) number of *Azotobacter* spp. PS activity was noted least (54.5%) among all the PGP traits and also in all three seasons as compared to other PGP traits (Fig. 4.2E).

4.3.2. PGP traits among soil bacteria from SMOF

Majority (93%) of bacterial isolates from SMOF displayed multiple PGP (MPGP) traits (T**able 4.4**).

Table 4.4. Number of PGP traits among bacterial isolates from SMOF

Organisms	**No. of isolates**	**No. of PGP traits (%)**			
		0	**1**	**2**	**>2**
Heterotrophs	149	16(10.7)	00	01(0.6)	132 (88.5)
Coliform	107	02(1.8)	00	02(1.8)	103 (96.2)
Pseudomonas spp.	132	10(9.3)	00	00	122 (92.4)
Azotobacter spp.	130	06(4.6)	00	00	124 (95.3)
Rhizobium spp.	132	06(4.5)	00	00	126 (95.4)
Total	650	40(6.1)	00	03(0.4)	607(93.3)

Coliforms, *Rhizobium* spp. and *Azotobacter* spp. emerged as top displayer of MPGP traits followed by *Pseudomonas* spp. and heterotrophs (**Table 4.4**). Out of the 650 total bacterial isolates examined, only 40 (6.1%) of them were not showed any PGP trait while none of the bacterial isolates displayed only one PGP trait and only 3 of them showed two PGP traits.

4.3.3. MPGP traits among bacteria from SMOF

Different bacterial groups showed MPGP traits are depicted in **fig. 4.3**. Overall large number of isolates representing different bacterial groups showed five MPGP traits followed by four and six MPGP traits. Over 45% nitrogen fixer representing both *Rhizobium* spp. and *Azotobacter* spp. showed five MPGP traits. Similarly *Pseudomonas* spp. (44.6%) and coliforms (38.4%) displayed six and four MPGP traits, respectively.

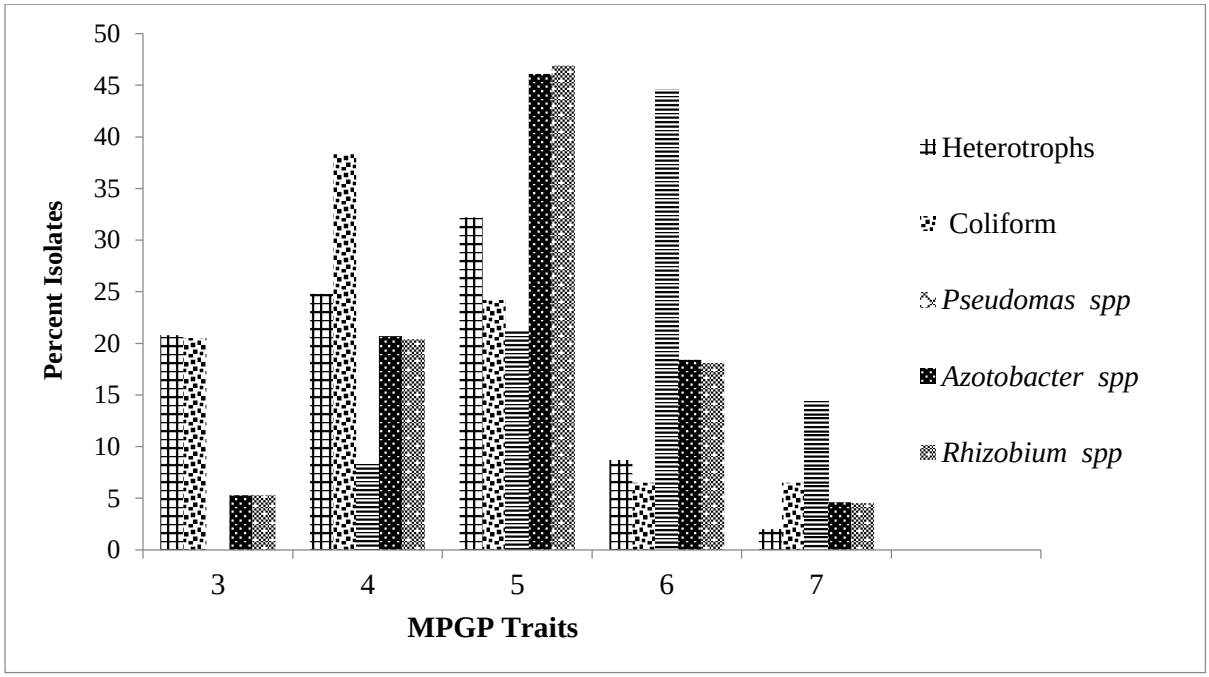


Figure 4. 3. Distribution of MPGP traits among different bacterial groups

4.3.4. Tolerance to salt among bacteria from SMOF

Bacteria isolated from soil of SMOF were studied for tolerance to salt and results are given in Table 4.5. Majority (74%) of isolates were tolerant to 5% salt whereas 25% of them were tolerant to 10% salt. Only three bacterial isolates representing two coliforms and one isolate of

Azotobacter spp. were tolerant to 20% salt. Expect for *Azotobacter* spp. large number (>78%) of others organisms displayed tolerance to 5% salt (**Table 4.5**). However, tolerance to 10% of salt was highest among *Azotobacter* spp. (48%) as compared to other bacterial isolates.

Table 4.5. Salt tolerance among bacterial isolates from SMOF

Organisms	**No. of isolates**	**No. of organisms tolerant to Salt (%)**		
		5	**10**	**20**
Heterotrophs	149	125(83.8)	24(16.1)	0
Coliform	107	82(76.6)	23(21.4)	2(1.8)
Pseudomonas spp.	132	106(80.3)	26(19.6)	0
Rhizobium spp.	132	103(78.0)	29(21.9)	0
Azotobacter spp.	130	66(50.7)	63(48.4)	1(0.7)
Total	650	482(74.0)	165(25.3)	3(0.4)

Richness of their functional characteristics was further revealed by their tolerance salinity and wide range of pH. Soil salinity and extreme pH are matter of serious concern for agricultural productivity. Use of salt tolerant PGPB is an effective approach to enhance growth and tolerance of various crops under salt stress conditions (**Sharma *et al.*, 2016**). In the present study all isolates from organic farm were tolerant to >5 % NaCl and wide range of pH. Biologically nitrogen fixing organisms have a very important role in any agro-ecological system due to their ability to convert atmospheric nitrogen into fertilizer. In the present study we obtained major (96-97%) constituent of bacterial population of nitrogen fixers *Azotobacter* spp. and *Rhizobium* spp with multiple PGP traits and tolerance to salinity and wide range of pH.

4.3.5. Tolerance to pH among bacteria from SMOF

Tolerance to pH among bacterial isolates from SMOF is given in **Table 4.6**. From the results it is clear that bacterial isolates from organic farm displayed tolerance to variable range of pH. Of the 650 bacterial isolates studied for their tolerance to pH range, 485 (74.6%) of them displayed

tolerance to wide range of pH 5-9 (**Table 4. 6**). However, at neutral pH 7 very few 53(8.1%) of the isolates exhibited growth. The maximum no of bacterial group's *viz.,* heterotrophs followed by *Pseudomonas* spp. and least by coliform was found at optimum pH in range of 5-9.

Table 4.6. Tolerance to pH among bacterial isolates from SMOF

Organisms	**No. of isolates**	**No. of organisms tolerant to pH (%)**						
		5-9	**5-8**	**6-9**	**6-7**	**7**	**7-9**	**7-8**
Heterotrophs	149	123(82.0)	0	24(16.1)	0	0	0	2(1.3)
Coliform	107	83(77.5)	1(0.9)	5(4.6)	2(1.8)	5(4.6)	3(2.8)	8(7.4)
Pseudomonas spp.	132	107(81.0)	0	6(4.5)	0	9(6.8)	4(3.0)	6(4.5)
Rhizobium spp.	132	88(66.6)	0	1(0.7)	3(2.2)	33(25.0)	5(3.7)	2(1.5)
Azotobacter spp.	130	84(64.6)	0	23(17.6)	0	7(5.3)	3(2.3)	13(10.0)
Total	650	485(74.6)	1(0.1)	59(9.0)	5(0.7)	54(8.3)	15(2.3)	31(4.7)

4.3.6. Comparison of Production of HCN, SD and PS among different bacteria

Comparison of production of HCN and SD among different PGPB is given in **table 4.7 and fig 4.4** respectively. Overall 48% agreements were noted between production of HCN and SD. Agreements between different groups of PGPB ranged between 26 to 70 %; being highest (70%) among coliform and least (26%) in *Azotobacter* spp.

Table 4.7. Comparison of production of HCN with SD in different PGPB

Organism	No. of isolates	HCN + SD+	HCN- SD-	Agreement between activity (%)
Heterotrophs	149	10	74	56
Coliform	107	3	72	70*
Pseudomonas spp.	132	55	5	45
Rhizobium spp.	132	22	51	55
Azotobacter spp.	130	16	19	26**
Total	650	106	221	48

HCN= production of hydrogen cyanide, SD= siderophore production

Comparison of production of HCN and PS among different PGPB is given in **table 4.8 and fig 4.5** respectively. Overall 57% agreements were noted between production of HCN and PS. Agreements between different groups of PGPB ranged between 46 to 70 %; being highest (70%) among *Azotobacter* spp. and least (46%) in *Rhizobium* spp.

From the results presented in this study it is clear that production of HCN is comparable with PS activity (57 % agreement). Similar production of HCN is comparable with SD except for *Azotobacter* sp.

Volatile compound HCN produced by soil bacteria is mainly considered as biocontrol agent especially against phytopathogenic fungi (**Ramette *et al.*, 2003; Ahmad *et al.*, 2008; Rezzonico *et al.*, 2007; Siddiqui *et al.*, 2006**). However, Contradictory to the role of HCN as biocontrol agent, several researchers concluded that HCN is hardly a universal biocontrol agent (**Alström and Burns, 1989; Pal *et al.*, 2000; Kremer and Souissi, 2001; Rudrappa *et al.*, 2008; Blom *et al.*, 2011**). In a recent study, **Rijavec and Lapanje (2016)** have clearly shown that there is no correlation between the amount of HCN produced by a particular strain and its ability to inhibit the growth of phytopathogenic bacteria or fungi. On the other hand they proposed a new role for HCN production by rhizospheric bacteria that is HCN increases

phosphate availability for rhizobacteria and plant hosts especially in oligotrophic alpine environments.

Microbes play an important role in the acquisition and transfer of nutrients in soil (**Richardson, 2001**). Therefore, the utilization of soil microbes to activate minerals and enhance nutrient uptake in plants has attracted increasing attention in sustainable agriculture (**Fayez and Mahmoud, 2006**).

Recent finding by **Rijavec and Lapanje (2016)** showed that the main contribution of HCN is in the sequestration of metals and indirectly increasing the availability of phosphate, thus consequently increasing nutrients availability has introduced a paradigm shift in understanding the role of biogenic cyanide in plant growth promotion activity.

Table 4.8. Comparison of production of HCN with PS in different PGPB

Organism	**No. of isolates**	**HCN + PS+**	**HCN- PS-**	**Agreement between activity (%)**
Heterotrophs	149	6	85	61
Coliform	107	2	63	60
Pseudomonas spp.	132	41	20	46
Rhizobium spp.	132	12	52	48
Azotobacter spp.	130	12	80	70
Total	650	73	300	57

HCN= production of hydrogen cyanide, PS= phosphate solubilization activity

	HCN +	HCN -	
SD +	10	34	44
SD -	31	74	105
	41	108	149

Heterotrophs

	HCN +	HCN -	
SD +	22	54	76
SD -	05	51	56
	27	105	132

Rhizobium spp.

	HCN +	HCN -	
SD +	03	32	35
SD -	0	72	72
	03	104	107

Coliform

	HCN +	HCN -	
SD +	16	83	99
SD -	12	19	31
	48	102	130

Azotobacter spp.

	HCN +	HCN -	
SD +	55	56	111
SD -	26	05	105
	81	51	132

Pseudomonas spp.

	HCN +	HCN -	
SD +	106	259	365
SD -	74	211	285
	180	470	650

All data

Figure 4.4. Contingency table for HCN and SD activities in different organism from organic farm

		HCN	
	+	-	
PS +	06	23	29
PS -	35	85	120
	41	108	149

Heterotrophs

		HCN	
	+	-	
PS +	12	53	65
PS -	15	52	67
	27	105	132

Rhizobium spp.

		HCN	
	+	-	
PS +	02	41	43
PS -	0 1	63	64
	03	104	107

Coliform

		HCN	
	+	-	
PS +	12	22	34
PS -	16	80	96
	28	102	130

Azotobacter spp.

		HCN	
	+	-	
PS +	41	31	72
PS -	40	20	60
	81	51	132

Pseudomonas spp.

		HCN	
	+	-	
PS +	73	170	243
PS -	107	300	407
	180	470	650

All data

Figure 4.5. Contingency table for HCN and PS activities in different organism from organic farm

4.4. Selection of potential PGPB

Twenty nine (29) potential PGPB were selected based on positivity to ACCD, IAA, siderophore, tolerance to salinity and pH for further studies. These potential PGPB were characterized on the basis of morphological and biochemical as described in Bergy's Manual of Determinative Bacteriology in **table 4.9.**

Table 4.9. Morphological and biochemical characterization of potential PGPB

	Gram negative						**Gram positive**
Morphological							
	***Enterobacter* sp.**	***Escherichia hermannii***	***Inqilinus* sp.**	***Pseudomonas* sp.**	***Rhizobium* sp.**	***Azotobacter* sp.**	***Bacillus subtillis***
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	Motile	Motile	Non motile	Motile	Motile	Motile	Motile
Spore	Negative	Negative	Negative	Negative	Negative	Negative	Positive
Biochemical characteristics							
Oxidase	-	-	-	+	+	+	+
OF	F	F	F	O	O	O	F
I	-	+	-	+	-	+	-

M	+	+	+	-	+	+	-
VP	+	-	+	-	+	+	+
Citrate	+	-	-	+	-	-	+
H_2S	-	-	-	-	+	+	-
Glucose	AG	A	A	G	G	A	A
Sucrose	G	G	A	G	G	A	A
Lactose	G	A	A	G	G	A	A
Maltose	A	A	A	G	G	A	A
GL	-	+	-	-	-	-	+

OF= Oxidative/fermentative, I= Indole, M= Methyl-Red, V= Voges-Proskauer, C= citrate, - = negative, + = positive, GL= Gelatin liquidation

4.4.1 Molecular identification and phylogenetic analysis of potential PGPB using 16s ribosomal gene markers

In case of 16s ribosomal gene analysis, 16s ribosomal RNA gene-specific primers were used and they amplified the genomic DNA of 29 isolates and produced ~1500-bp amplicons. All the 29 isolates shared more than 99 % nucleotide sequence similarity with 16s rRNA gene sequences of eight different bacterial species, available in NCBI Gen-Bank. Sequence obtained reported in present study has been also deposited in the NCBI Gen Bank nucleotide sequence database under the different accession numbers (**Table 4.10**). Molecular identification based on sequences of 16s rRNA gene confirmed that the isolates belonging to eight different species viz., *Enterobacter* sp. (15), *Pseudomonas* sp. (04), *Rhizobium* sp.(03), *Azotobacter* sp.(02), and other sp. *Erwinia* sp.(01), *Inqilinus* sp.(01), *Escherichia hermannii (01)* and *Bacillus subtillis* (02) respectively (**Table 4.10 and fig 4.6**).

Caldwell *et al.*, 2015 reported the differences in the prokaryotic soil microbiology of three Brazilian coffee farms: one practicing intensive farming, one practicing organic farming, and one undergoing a transition from intensive to organic practices. Two groups of prokaryotes associated with the nitrogen cycle, the archaeal genus Candidatus Nitrososphaera and the bacterial order Rhizobiales were found to be abundant in organic farms.

Organic farming have a more stable microflora and the uniformity of the bacterial community structure. Organic agriculture significantly increased the abundance of some nutrition-related bacteria, while reducing some of the abundance of acid and alkali resistant bacteria. Under organic farming, several predominant guilds and major bacterial lineages (Rhizobiales, Thiotrichaceae, Micromonosporaceae, Desulfurellaceae and Myxococcales) contributing to nutrient (C, N, S and P) cycling were enriched, whereas the relative abundances of acid and alkali resistant microorganisms (Acidobacteriaceae and Sporolactobacillaceae) were increased under conventional farming practices.

Table 4.10. Molecular identification of potential PGPB with their NCBI Accession no

Strain No.	Organism	NCBI Accession no
PR1	*Enterobacter cloacae*	KP226566
PR2	*Enterobacter cloacae*	KP226567
PR3	*Enterobacter cloacae*	KP226568
PR4	*Enterobacter cloacae*	KP226569
PR5	*Enterobacter* sp.	KP226571
PR6	*Erwinia* sp.	KP226572
PR7	*Enterobacter* sp.	KP226581
PR8	*Enterobacter* sp.	KP226573
PR9	*Enterobacter* sp.	KP226574
PR10	*Inqilinus* sp.	KP226575
PR11	*Enterobacter* sp.	KP226576
PR12	*Enterobacter* sp.	KP226582
PR13	*Enterobacter* sp.	KP226577
PR14	*Enterobacter* sp.	KP226570
PR15	*Escherichia hermannii*	KP226578

PR16	*Enterobacter* sp.	KP226579
PR17	*Enterobacter* sp.	KP226580
PR19	*Azotobacter nigricans*	KP966496
PR20	*Azotobacter chroococcum*	KP966500
PR21	*Pseudomonas* sp.	KP966497
PR22	*Pseudomonas* sp.	KP966501
PR23	*Rhizobium* sp.	KP966502
PR24	*Rhizobium* sp.	KP966498
PR25	*Pseudomonas* sp.	KP966506
PR26	*Rhizobium* sp.	KP966507
PR28	*Enterobacter soli*	KP966503
PR29	*Pseudomonas chlororaphis*	KP966504
PR30	*Bacillus subtillis*	KP966505
PR31	*Bacillus subtillis*	KP966499

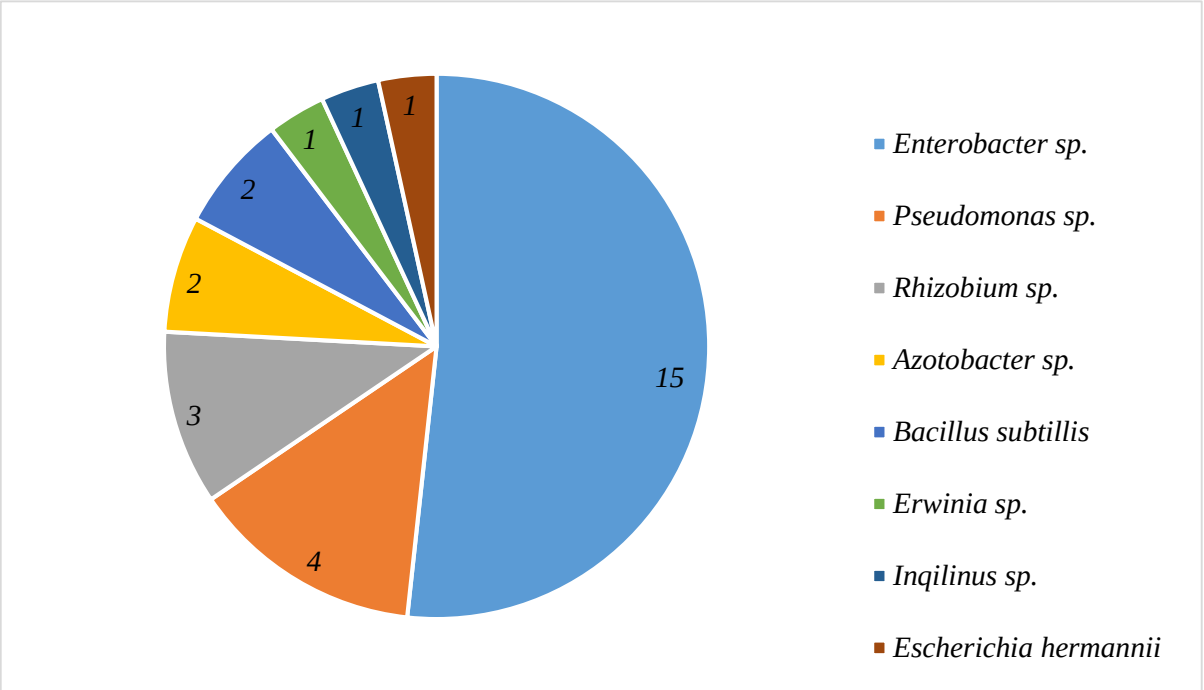


Figure 4.6. Plant growth promoting bacteria (PGPB) isolated from organic farm

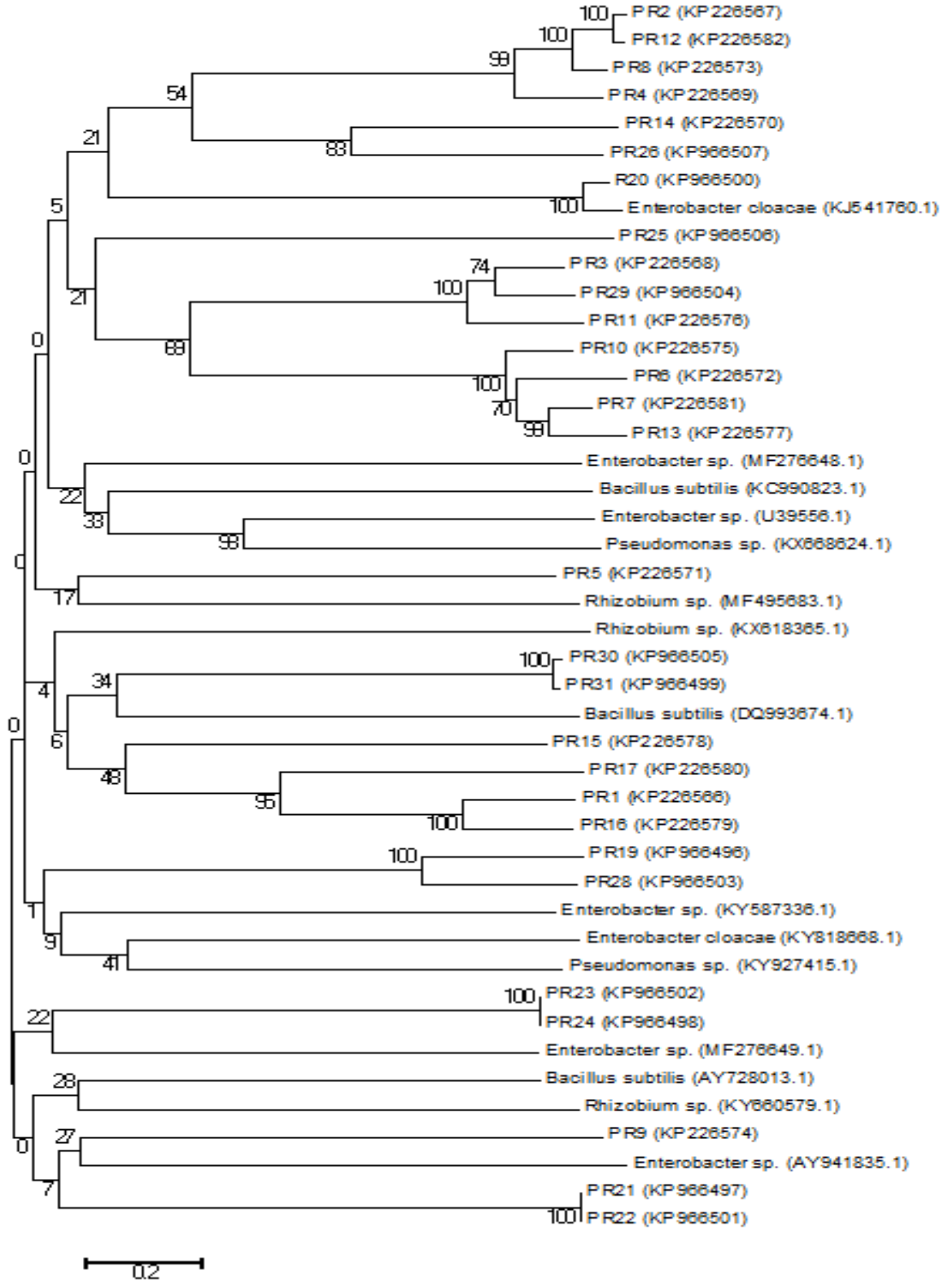


Figure 4.7. Phylogenetic tree (N-J tree) showing relationship between isolates and other type strains based on 16S rDNA sequences for structure analysis; bootstrap values derived from 1000 replicates

The result of the phylogenetic analysis based on the 16s rRNA gene sequence of 29 bacterial isolates is shown in **Fig. 4.7.** A phylogenetic tree was constructed using the 16S rRNA gene sequences of the isolates and type strains obtained from the NCBI GenBank database. Phylogenetic tree exhibited a great diversity among the isolates. The tree was grouped into different clusters. Majority of the *Enterobacter* isolates share the common clade along with the type strains *Enterobacter cloacae* (KJ541760) and *Enterobacter* sp. (AY941835) with the sequence similarity >98%. Rhizobium and Azobotobacter isolates also clustered with the type strain *Enterobacter cloacae* (KJ541760), indicating the events of horizontal gene transfer within the different groups of bacteria. *Bacillus subtillis* isolates (PR30 and PR31) were grouped with the Bacillus subtillis type strain (DQ993674) in the same cluster, showed 100% sequence similarity with each other. *Pseudomonas* isolates (PR21 and PR22) shared the same cluster with 100% sequence similarity.

4.4.2. Characterization of PGP traits among potential PGPB

PGP traits of potential PGPB are given in **table 4.11**. Majority of them found positive for production of NH_3, IAA, ACCD and catalase, least for production of HCN, SD and PS activity except PR 31. The isolates PR22 (*Pseudomonas* spp.), PR23 and PR24 (*Rhizobium* spp.) were found to be positive for the test of catalase, NH_3, IAA, ACCD, HCN, SD and PS activity.

It has been known that PGPB in the rhizosphere can affect plants directly by promoting plant growth or indirectly by either inhibiting plant pathogens or increasing plant resistance to pathogens (Biocontrol-PGPB) (**van Loon, 2007; Yang *et al.*, 2009**). Agriculture has greatly benefitted from this knowledge and PGPB inoculants have been used extensively to increase farm productivity (**Rodriguez *et al.*, 2006**). Classic examples of PGPB that benefit plants by fixing nitrogen in the genera *Rhizobium, Agrobacterium, Acetobacter* and *Azospirillum* were detected in fairly low relative abundance in our samples, even though the families in which these microbes belong to had some of the highest relative abundances (# of sequences in the family divided by all sequences in each sampling site) compared to all other families in our database. Other studies have shown that rhizosphere bacteria produce more IAA than bulk soil bacteria (**Khalid *et al.*, 2004**), and in a recent study (**Costa *et al.*, 2014**) showed that this effect was also observed in endophytic bacteria, demonstrating high IAA production in the Enterobacteriaceae family (*Enterobacter, Escherichia, Grimontella, Klebsiella, Pantoea,* and *Rahnella*).

Table 4.11. PGP traits among potential PGPB

Strain No.	Organism	NCBI Accession no	PGP Traits							
			NH_3	HCN	SD	IAA	ACCD	PS	Chitinase	Catalase
PR1	*Enterobacter cloacae*	KP226566	+	+	-	3.0	+	+	-	+
PR2	*Enterobacter cloacae*	KP226567	+	+	-	4.0	+	+	-	+
PR3	*Enterobacter cloacae*	KP226568	+	+	-	10.0	+	+	-	+
PR4	*Enterobacter cloacae*	KP226569	+	+	-	9.0	+++	+	-	+
PR5	*Enterobacter* sp.	KP226571	+	+	+	24.0	+	+	-	+
PR6	*Erwinia* sp.	KP226572	+	-	+	5.5	+	+	-	+
PR7	*Enterobacter* sp.	KP226581	+	-	+	17.0	+	+	+	+
PR8	*Enterobacter* sp.	KP226573	+	-	+	15.0	+	+	-	+
PR9	*Enterobacter* sp.	KP226574	+	-	+	16.0	+	-	-	+
PR10	*Inqilinus* sp.	KP226575	+	+	+	7.0	+	+	-	+
PR11	*Enterobacter* sp.	KP226576	+	+	-	14.0	+	+	-	+

PR12	*Enterobacter* sp.	KP226582	+	+	-	17.5	+	+	-	+
PR13	*Enterobacter* sp.	KP226577	+	+	+	19.0	+	+	-	+
PR14	*Enterobacter* sp.	KP226570	+	-	-	13.0	+++	-	-	+
PR15	*Escherichia hermannii*	KP226578	+	-	+	21.0	+	-	-	+
PR16	*Enterobacter* sp.	KP226579	+	-	+	12.0	+	-	-	+
PR17	*Enterobacter* sp.	KP226580	+	-	+	17.0	+	-	-	+
PR19	*Azotobacter nigricans*	KP966496	+	+	+	22.0	+++	+	-	+
PR20	*Azotobacter chroococcum*	KP966500	+	-	+	21.5	+	+	-	+
PR21	*Pseudomonas* sp.	KP966497	+	+	+	11.0	+	-	-	+
PR22	*Pseudomonas* sp.	KP966501	+	+	+	8.5	+	+	+	+
PR23	*Rhizobium* sp.	KP966502	+	+	+	14.0	+	+	+	+
PR24	*Rhizobium* sp.	KP966498	+	+	+	16.0	+	+	+	+
PR25	*Pseudomonas* sp.	KP966506	+	-	+	25.5	+	+	-	+

PR26	*Rhizobium* sp.	KP966507	+	-	+	70.0	+	-	-	+
PR28	*Enterobacter soli*	KP966503	+	-	+	36.0	+	+	-	+
PR29	*Pseudomonas chlororaphis*	KP966504	+	-	-	63.0	+	+	-	+
PR30	*Bacillus subtillis*	KP966505	+	-	-	54.5	+	+	-	+
PR31	*Bacillus subtillis*	KP966499	-	+	-	50.5	-	-	-	-

production of ammonia = NH3, hydrogen cyanide = HCN, siderophore = SD, indole acetic acid = IAA, 1-aminocyclopropane- 1-carboxylate deaminase = ACCD , phosphorus solubilization activity= PS + =Presence, - = negative

4.4.3. Tolerant to environmental stress

Potential PGPB were studied for tolerant to environmental stress *viz*. salt, pH and catalase are given in **table 4.12**. Majority of isolates were tolerant to 10% salt and 5-9 pH whereas only three (PR4, PR14 and PR19) potential PGPB were tolerant to 20% salt. Two isolates (PR21 and PR31) were tolerant to 5% salt.

Table 4.12.Traits tolerant to environmental stress among potential PGPB

Strain No.	**Organism**	**NCBI Accession no**	**Tolerance**	
			Salt %	**pH**
PR1	*Enterobacter cloacae*	KP226566	10	7,8
PR2	*Enterobacter cloacae*	KP226567	10	7,8
PR3	*Enterobacter cloacae*	KP226568	10	7-9
PR4	*Enterobacter cloacae*	KP226569	20	5-9
PR5	*Enterobacter* sp.	KP226571	10	5-9
PR6	*Erwinia* sp.	KP226572	10	5-9
PR7	*Enterobacter* sp.	KP226581	10	6-9
PR8	*Enterobacter* sp.	KP226573	10	5-9
PR9	*Enterobacter* sp.	KP226574	10	7,8
PR10	*Inqilinus* sp.	KP226575	10	6-9
PR11	*Enterobacter* sp.	KP226576	10	5-9
PR12	*Enterobacter* sp.	KP226582	10	5-9
PR13	*Enterobacter* sp.	KP226577	10	5-9
PR14	*Enterobacter* sp.	KP226570	20	5-9
PR15	*Escherichia hermannii*	KP226578	10	6,7,9
PR16	*Enterobacter* sp.	KP226579	10	7,8
PR17	*Enterobacter* sp.	KP226580	10	7-8
PR19	*Azotobacter nigricans*	KP966496	20	5-9
PR20	*Azotobacter chroococcum*	KP966500	10	5-9
PR21	*Pseudomonas* sp.	KP966497	10	5-9
PR22	*Pseudomonas* sp.	KP966501	5	5-9
PR23	*Rhizobium* sp.	KP966502	10	5-9
PR24	*Rhizobium* sp.	KP966498	10	7-9

PR25	*Pseudomonas* sp.	KP966506	10	5-9
PR26	*Rhizobium* sp.	KP966507	10	5-9
PR28	*Enterobacter soli*	KP966503	10	5-9
PR29	*Pseudomonas chlororaphis*	KP966504	10	5-9
PR30	*Bacillus subtillis*	KP966505	10	5-9
PR31	*Bacillus subtillis*	KP966499	5	5-8

4.4. Tolerance to trace elements

Result of tolerance to trace elements of potential PGPB are summarized in **table 4.13**. These isolates were tolerance to trace elements such as Zn, As, Al, Mo, Mn, Ag, Ni and Cr. Hg and Au proved to be toxic for the organism. High level of tolerance was detected for Al, Zn and Mo (>800µg/ml), Mn and Pb (>400 µg/ml), followed by Cr, Cu, Ag, As and Ni (100 µg/ml), Ag and Au (>2.5 µg/ml).

Table 4.13.Tolerance to trace elements among potential PGPB

Strain no	Organism	Tolerance to trace elements (µg/ml)											
		Hg	**Au**	**Cu**	**Ag**	**Cr**	**As**	**Ni**	**Mn**	**Pb**	**Al**	**Mo**	**Zn**
PR1	*Enterobacter cloacae*	2.5	25	100	100	200	100	200	400	400	800	800	3200
PR2	*Enterobacter cloacae*	2.5	25	100	100	100	100	100	400	400	800	400	1600
PR3	*Enterobacter cloacae*	2.5	25	100	100	100	100	100	400	400	800	400	1600
PR4	*Enterobacter cloacae*	2.5	12	100	100	200	200	100	400	400	800	800	3200
PR5	*Enterobacter* sp.	1.2	50	100	50	200	100	100	400	200	3200	800	800
PR6	*Erwinia* sp.	0.6	25	100	50	200	100	100	400	200	3200	800	800
PR7	*Enterobacter* sp.	0.6	25	50	50	200	100	100	400	200	3200	800	1600
PR8	*Enterobacter* sp.	0.6	50	50	50	200	100	100	400	200	3200	800	800
PR9	*Enterobacter* sp.	0.6	25	50	50	200	100	100	400	200	3200	800	1600
PR10	*Inqilinus* sp.	0.6	50	50	100	200	100	100	400	200	3200	800	800
PR11	*Enterobacter* sp.	0.6	50	50	50	200	100	50	400	200	3200	800	800
PR12	*Enterobacter* sp.	0.6	50	50	50	200	100	50	400	200	3200	800	1600
PR13	*Enterobacter* sp.	0.6	25	50	50	100	100	50	400	200	3200	800	800
PR14	*Enterobacter* sp.	1.2	12	50	50	200	50	200	400	400	3200	800	800
PR15	*Escherichia hermannii*	1.2	25	50	100	200	100	100	200	200	3200	800	800
PR16	*Enterobacter* sp.	1.2	50	50	100	200	100	100	400	200	3200	800	800

PR17	*Enterobacter* sp.	0.6	25	50	50	200	100	100	400	200	3200	800	1600
PR19	*Azotobacter nigricans*	2.5	25	100	100	200	50	200	200	400	3200	800	1600
PR20	*Azotobacter chroococcum*	0.6	25	50	50	200	100	100	400	200	3200	800	800
PR21	*Pseudomonas* sp.	1.2	50	50	50	200	100	100	400	200	3200	800	1600
PR22	*Pseudomonas* sp.	2.5	25	100	100	200	100	200	400	200	800	800	1600
PR23	*Rhizobium* sp.	2.5	12	100	200	200	100	100	400	400	800	800	1600
PR24	*Rhizobium* sp.	1.2	50	50	50	200	100	100	400	200	3200	800	1600
PR25	*Pseudomonas* sp.	0.6	25	50	50	200	100	100	400	200	3200	800	1600
PR26	*Rhizobium* sp.	0.6	25	50	50	200	100	100	400	200	3200	800	1600
PR28	*Enterobacter soli*	1.2	50	50	50	200	100	50	400	200	3200	800	1600
PR29	*Pseudomonas chlororaphis*	2.5	12	100	100	200	100	200	400	200	800	800	3200
PR30	*Bacillus subtillis*	2.5	12	100	200	200	100	100	400	400	200	800	800
PR31	*Bacillus subtillis*	2.5	25	100	200	200	100	100	400	400	200	800	800

4.4.4.1. Curing of trace elements tolerance among potential PGPB

Potential PGPB were screened for plasmid curing with trace elements are presented in **fig. 4.8**. Curing was observed in potential PGPB among trace elements expect *Erwinia* sp. *Enterobacter* sp. was highly cured with Ag, Cr, Ni, Mn and Mo. *Pseudomonas* sp. was highly cured with Mn and Mo.

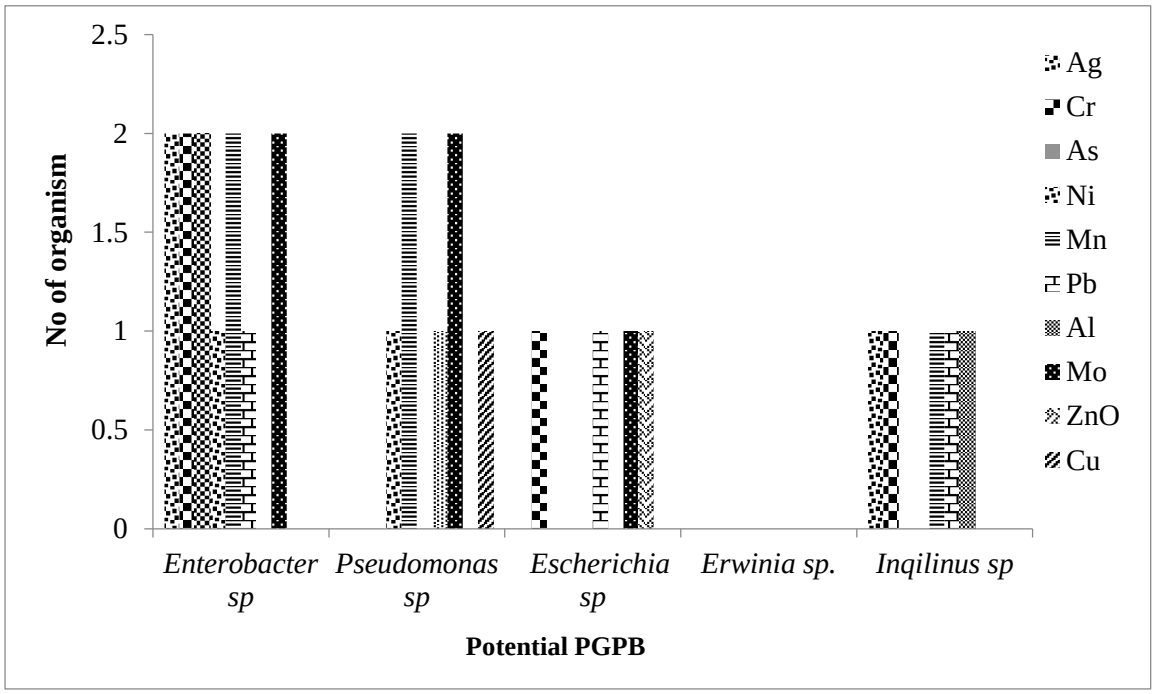


Figure 4.8. Curing of trace elements tolerance among different potential PGPB

4.4.4.2. Comparison of tolerance to trace elements and their curing among potential PGPB

Comparison of tolerance to trace elements and curing among potential PGPB are summarized in **fig. 4.9.** Potential PGPB showed highly tolerance to all trace elements expect Au. Potential PGPB cured with trace elements but least in numbers. Highly cured were noted in MO and Mn.

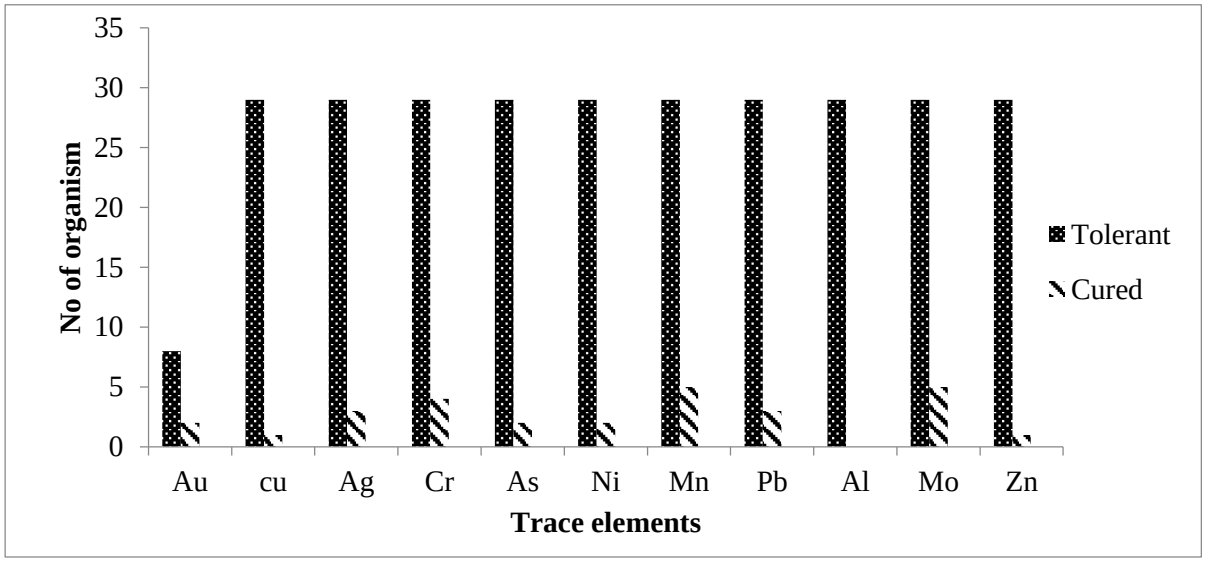


Figure 4.9. Comparison of tolerance to trace elements and their curing among potential PGPB

The result obtained in the current research revealed that the investigated bacterial isolates have PGP traits that can alleviate salt stress. The results are consist with previous results reported by **Siddike *et al.*, 2010; Mapelli *et al.*, 2013; and Dasele *et al.*, 2014**. The growth of PGPB strains from soil were affected by the salt and heavy metals. Indeed bacteria can protect plants from the effects of salt via production of IAA, ACC deaminase, abscisic acid, cytokinin, volatile organic compounds and exopolysaccharides (**Kim *et al.*, 2014; Forni *et al.*, 2017**).

With respect to soil salinity, bacteria carrying traits for phosphate solubilization, synthesis of siderophores, exopolysaccharides and indole acetic acid (IAA) and with high ACC-deaminase enzyme activity were shown to be effective PGPB (**Nadeem *et al.*, 2010; Upadhyay *et al.*, 2011; Glick, 2014**).

4.5. Antibiotic susceptibility of potential PGPB

Antibiotic susceptibility of potential PGPB is being indicated in the **table 4.14**. Majority of them were resistant to all antibiotics. Potential PGPB PR9, PR12, PR13, PR25 and PR26 were resistance to all antibiotic comparison to others PGPB.

Table 4.14. Antibiotic susceptibility of potential PGPB

Strain no	Organism	Antibiotic resistance (µg/ml)													Antibiotic Resistance patterns
		Cp (20)	A (20)	Cf (20)	NA (20)	NE (20)	S (20)	V (20)	K (20)	Rf (20)	C (30)	TM (20)	T (30)	G (10)	
PR1	*Enterobacter cloacae*	S	R	S	R	R	R	S	R	R	R	R	R	R	MAR
PR2	*Enterobacter cloacae*	S	R	S	R	R	R	S	R	S	R	R	R	R	MAR
PR3	*Enterobacter cloacae*	S	R	S	R	R	R	S	S	S	R	R	R	S	MAR
PR4	*Enterobacter cloacae*	S	S	S	R	S	R	S	R	S	R	S	R	R	MAR
PR5	*Enterobacter* sp.	R	S	R	R	S	S	R	R	S	R	R	S	R	MAR
PR6	*Erwinia* sp.	R	R	R	R	S	R	R	R	S	R	R	R	R	MAR
PR7	*Enterobacter* sp.	R	R	R	R	R	R	R	S	R	R	R	R	R	MAR
PR8	*Enterobacter* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR9	*Enterobacter* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR10	*Inqilinus* sp.	R	R	R	R	S	R	R	R	S	R	R	R	R	MAR
PR11	*Enterobacter* sp.	R	R	R	R	S	R	R	R	S	R	R	R	R	MAR
PR12	*Enterobacter* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR13	*Enterobacter* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR14	*Enterobacter* sp.	R	R	R	R	S	R	R	R	R	R	R	R	R	MAR

PR15	*Escherichia hermannii*	R	R	R	R	S	R	R	R	R	R	R	R	S	MAR
PR16	*Enterobacter* sp.	R	R	R	R	S	R	R	R	R	R	R	R	R	MAR
PR17	*Enterobacter* sp.	R	R	R	S	R	R	R	R	R	R	R	R	R	MAR
PR19	*Azotobacter nigricans*	R	R	R	R	S	R	R	R	S	R	R	R	R	MAR
PR20	*Azotobacter chroococcum*	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR21	*Pseudomonas* sp.	R	R	R	R	S	R	R	R	R	R	R	R	S	MAR
PR22	*Pseudomonas* sp.	S	R	S	R	R	R	S	R	R	R	R	R	R	MAR
PR23	*Rhizobium* sp.	S	R	R	R	R	R	S	R	S	R	R	R	R	MAR
PR24	*Rhizobium* sp.	R	R	R	R	S	R	R	R	R	R	R	R	R	MAR
PR25	*Pseudomonas* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR26	*Rhizobium* sp.	R	R	R	R	R	R	R	R	R	R	R	R	R	MAR
PR28	*Enterobacter soli*	R	R	R	R	S	R	R	R	S	R	R	R	R	MAR
PR29	*Pseudomonas chlororaphis*	S	R	S	R	R	R	S	R	S	R	R	R	R	MAR
PR30	*Bacillus subtillis*	S	R	R	R	R	R	S	R	S	R	R	R	R	MAR
PR31	*Bacillus subtillis*	S	R	R	R	S	S	S	R	S	R	R	R	R	MAR

R= Resistance, S= Sensitive, Cephalexin =Cp, Ampicillin=A, Cephataxime =Cf, Nalidixic =NA, Neomycin =Ne, Streptomycin =S, Vancomycin =V, Kanamycin =K, Rifampicin= Rc, chloramphenicol= C, Trimethoprim=TM, Tetracycline=T, Gentamycin=G

4.5.1. Curing of antibiotic resistance among potential PGPB

Curing of antibiotic resistance among potential PGPB are given in **fig 4.10**. Curing was observed in different PGPB among antibiotics expect *Escherichia* sp. *Enterobacter* sp. and *Pseudomonas* sp. were highly cured with K, S and Rf.

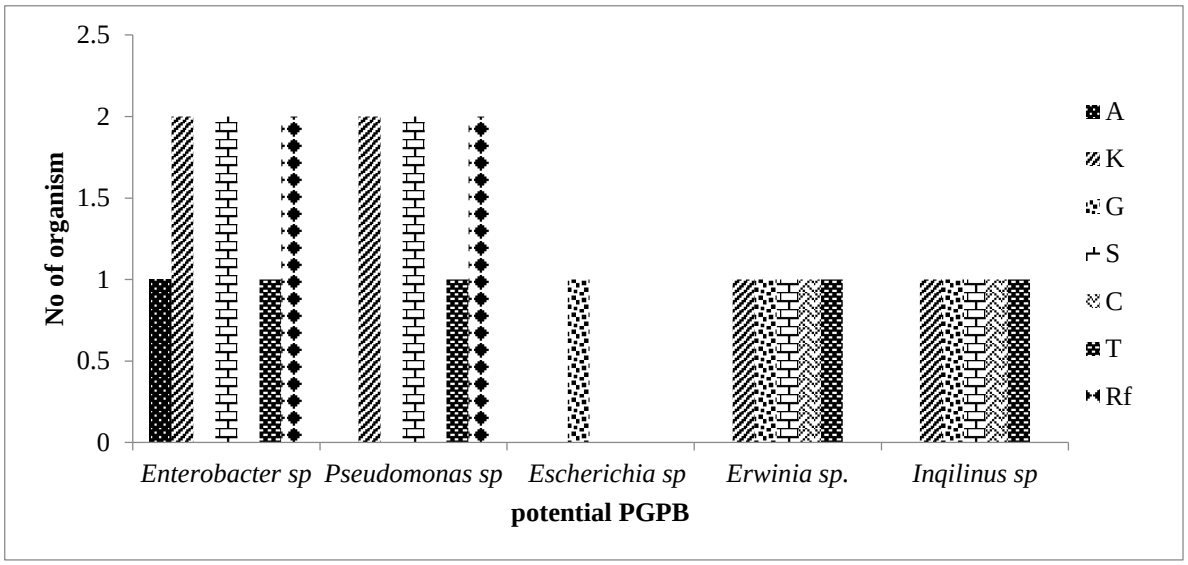


Figure 4.10. Curing of antibiotic resistance among different potential PGPB

4.5.2. Comparison of antibiotic resistance and curing of potential PGPB

Comparison of antibiotic resistance and curing of potential PGPB are given in **fig. 4.11**. < 20 potential PGPB showed high resistance to all antibiotic but least cured with K, G, S, C, T, TM, RF and A. Highly cured was noted with K and low in A.

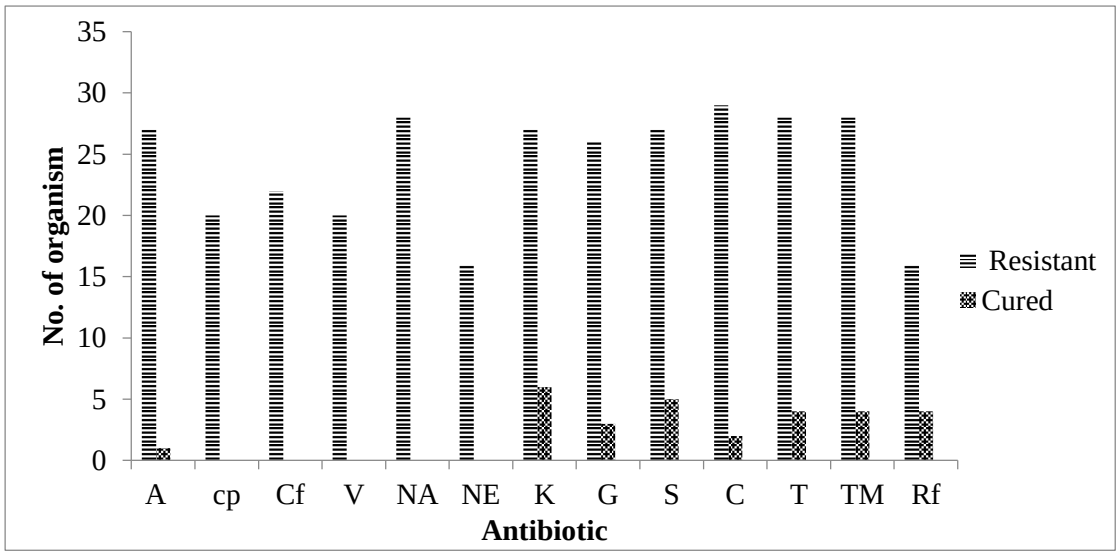


Figure 4.11. Comparison of antibiotic resistance and their curing of potential PGPB

4.5.3. Curing of ACCD among potential PGPB

Curing of ACCD among potential PGPB are given in **fig. 4.12**.Curing was observed in different PGPB among ACCD expect *Erwinia* sp. and *Inqilinus* sp. *Enterobacter* sp. was highly cured with ACCD comparison other potential PGPB.

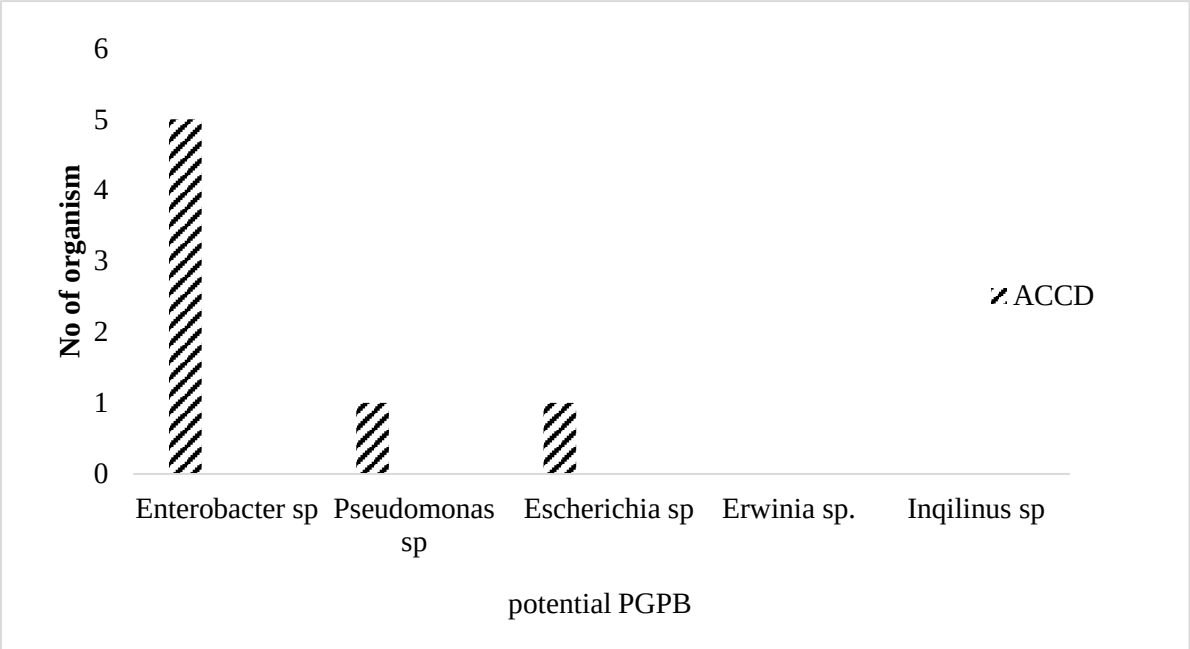


Figure 4.12. Curing of ACCD among different potential PGPB

4.4.5.4. Comparison of ACCD activity and curing of potential PGPB

Comparison of ACCD activity and curing of potential PGPB are presented in **fig. 4.13**. 100% ACCD activity was noted in potential PGPB but plasmid curing was noted in 7 organisms.

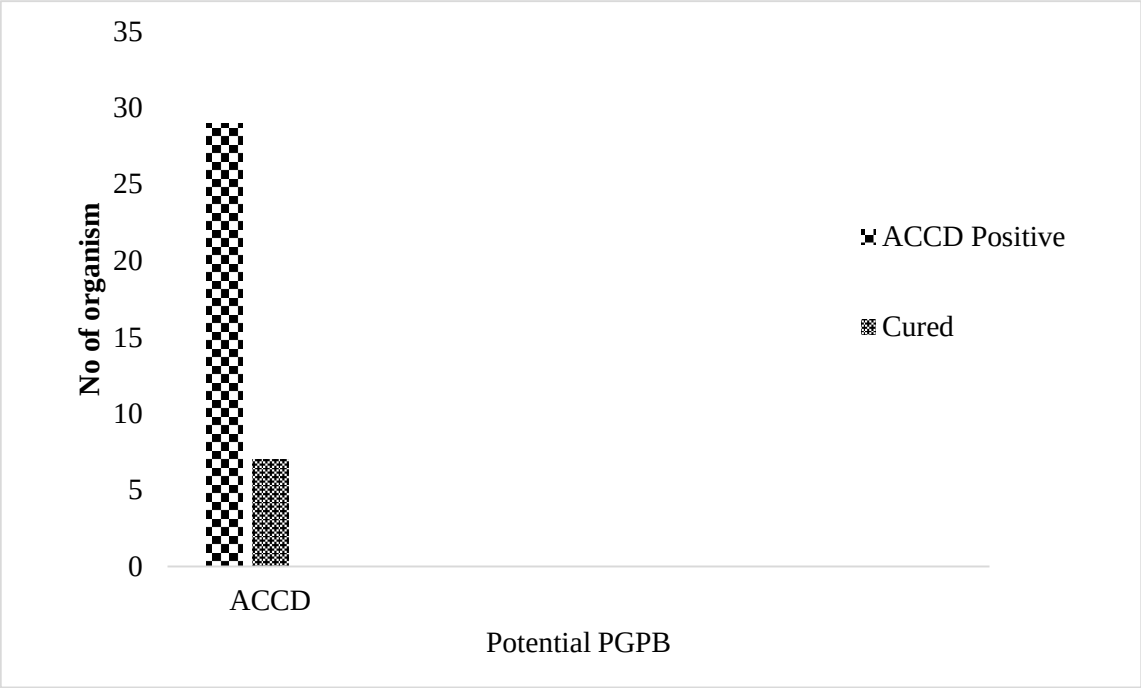


Figure 4.13. Comparison of ACCD activity and their curing among potential PGPB

These findings further suggest that the PGPB having resistance to antibiotics could be better utilized by inoculating these bacterial species to the rhizosphere of crops growing on contaminated soil with antibiotics. It is interesting to record heavy metal and antibiotic resistance from the PGPB isolated from an organic farm. The possible sources of antibiotic exposure of organic farm isolated PGPB could be the application of FYM ingredients including animal faeces (cow dung, cow urine, excreta from fowl, pet dogs, pigs etc.) from antibiotic administered domestic animals, leaves or plants sprayed with antibiotics, irrigation water contaminated with antibiotic compounds. Furthermore, several incidences related to linkage of resistance characteristics between heavy metals and antibiotics of bacterial plasmids are reported (**Ramteke, 1997; Ghosh *et al.*, 2000; Verma *et al.*, 2002; Tewari *et al.*, 2003; Siddiqui *et al.*, 2005; Ramteke *et al.*, 2012**). The present result of 100% curing of kanamycin and streptomycin indicate that these antibiotic resistance factors are purely plasmid borne and the acridine orange treatment completely eliminated the plasmids bearing the resistance factors. The 70-80% curing recorded for tetracycline, gentamycin antibiotics due to acridine orange

treatment suggests the probable intercalation of curing agent with plasmid DNA or possibility of the formation of A+T region specific antibiotic resistance factor-acridine orange adducts in the plasmid DNA (**Geoffrey *et al.*, 1978**) and resultant elimination of these regions in the self-replicating plasmids. Such instances of plasmid curing by acridine orange and consequent selective interferences in the replication of bacterial plasmids are already reported earlier (**Salisbury *et al*., 1972**).

4.6 Detailed studies with high salt tolerant (20%) PGPB (STPGPB)

On the basis of tolerance to high salt (20%) concentration the following organism were selected for further detailed studies:

1. *E. cloacae* (KP226569) (PR4)

2. Enterobacter sp. (KP226570) (PR14)

*3. A. nigricans (*KP966496) (PR19)

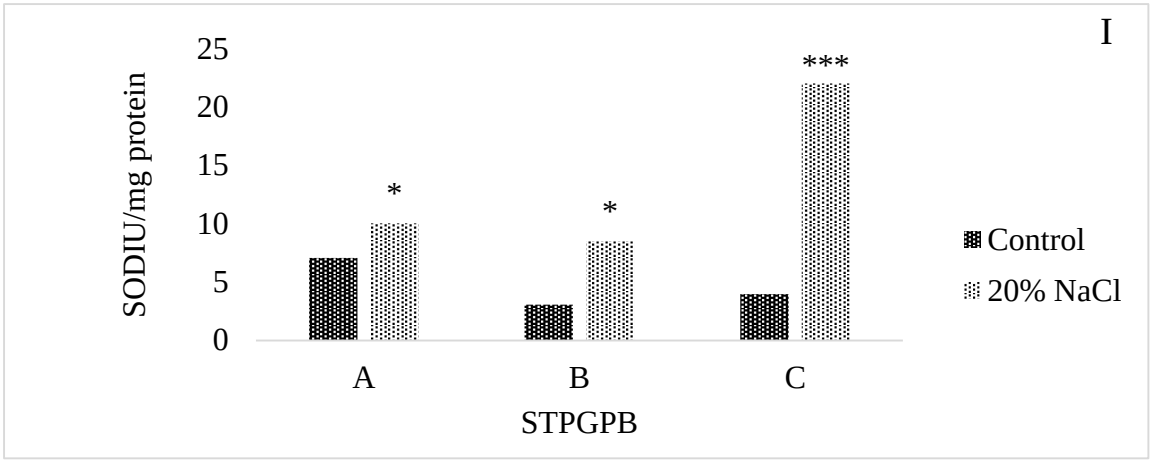
4.6.1. Antioxidant enzyme activity among STPGPB under abiotic stress

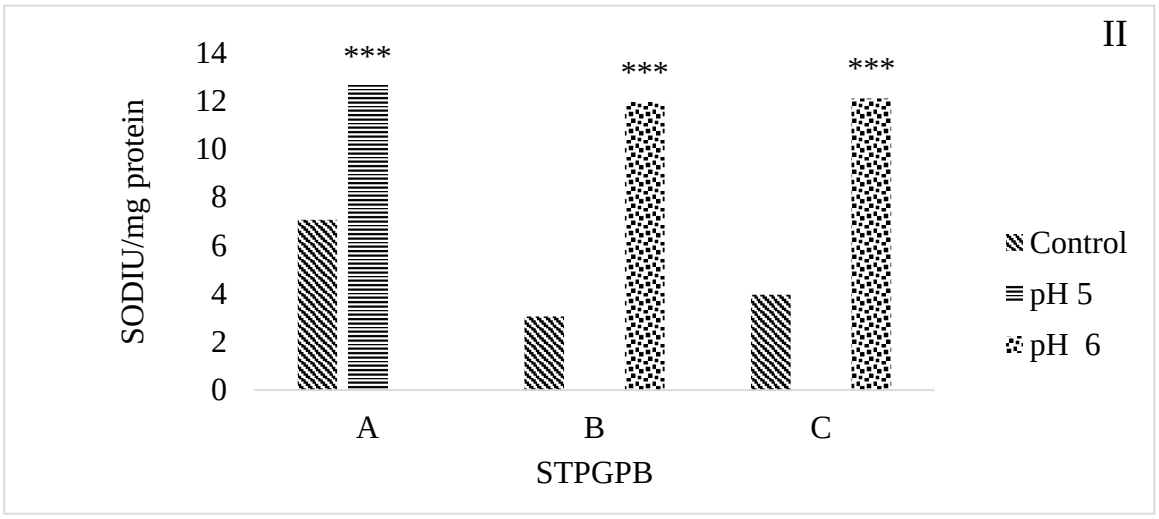
Three STPGPB tolerant to 20% NaCl viz. *E. cloacae* (KP226569), *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) where studied for antioxidant enzyme activity *viz*. SOD, CAT and GSH.

4.6.1.1 Antioxidant enzyme SOD activity by STPGPB under abiotic stress

The results of three STPGPB studied for antioxidant enzyme SOD under abiotic stresses viz 20% salt, acidic pH (5-6) and alkaline pH are presented in **fig 4.14**. All the three STPGPB expressed significant increase in SOD activity when subjected to three abiotic stresses (**fig 4.14**).

When subject to 20% NaCl *A. nigricans* (KP966496) expressed significant high ($p<0.001$) activity of SOD. However, both the species of *Enterobacter* showed significant ($p<0.05$) expression of SOD (**fig 4.14. I**). Under abiotic stress of acidic pH all the three organism expressed significantly high ($p<0.001$) SOD activity (**fig 4.14.II**). When subjected to alkaline pH 9 *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) expressed significantly high ($p<0.001$) SOD activity, whereas *E. cloacae* (KP226569) did not show significant SOD activity (**fig 4.14.III**).





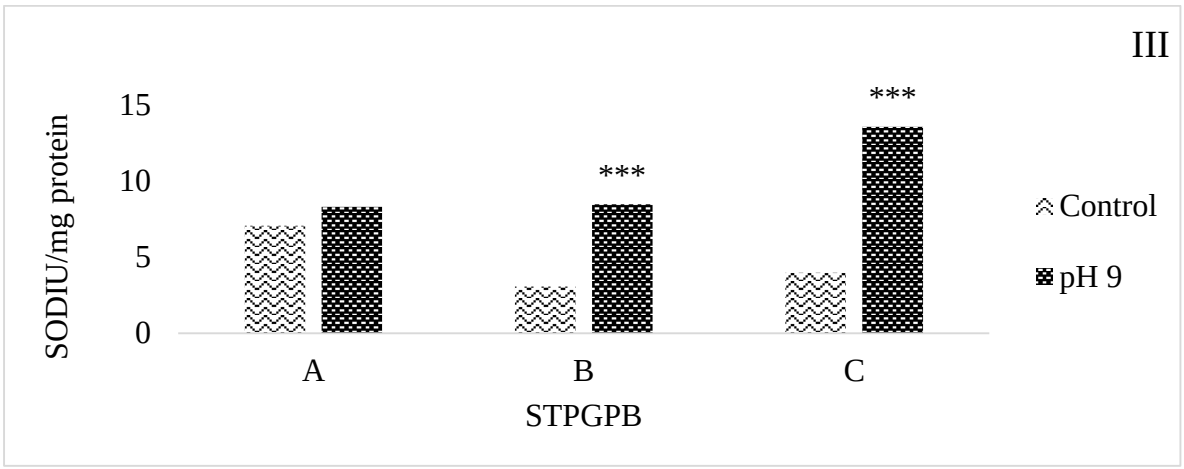


Figure 4.14. Comparison of SOD activity of STPGPB under abiotic stress

I= 20% NaCl, II = acidic pH (5 and 6), II= alkaline pH (9), A= *E. cloacae* (KP226569), B= *Enterobacter* sp. (KP226570), C= *A. nigricans* (KP966496), *p<0.05; **P<0.01; ***p<0.001

4.6.1.2. Antioxidant enzyme CAT activity among STPGPB under abiotic

All the three organisms were studied for antioxidant enzyme CAT activity under abiotic stresses viz 20% NaCl, acidic pH (5-6) and alkaline pH and results are presented in **fig 4.15**.

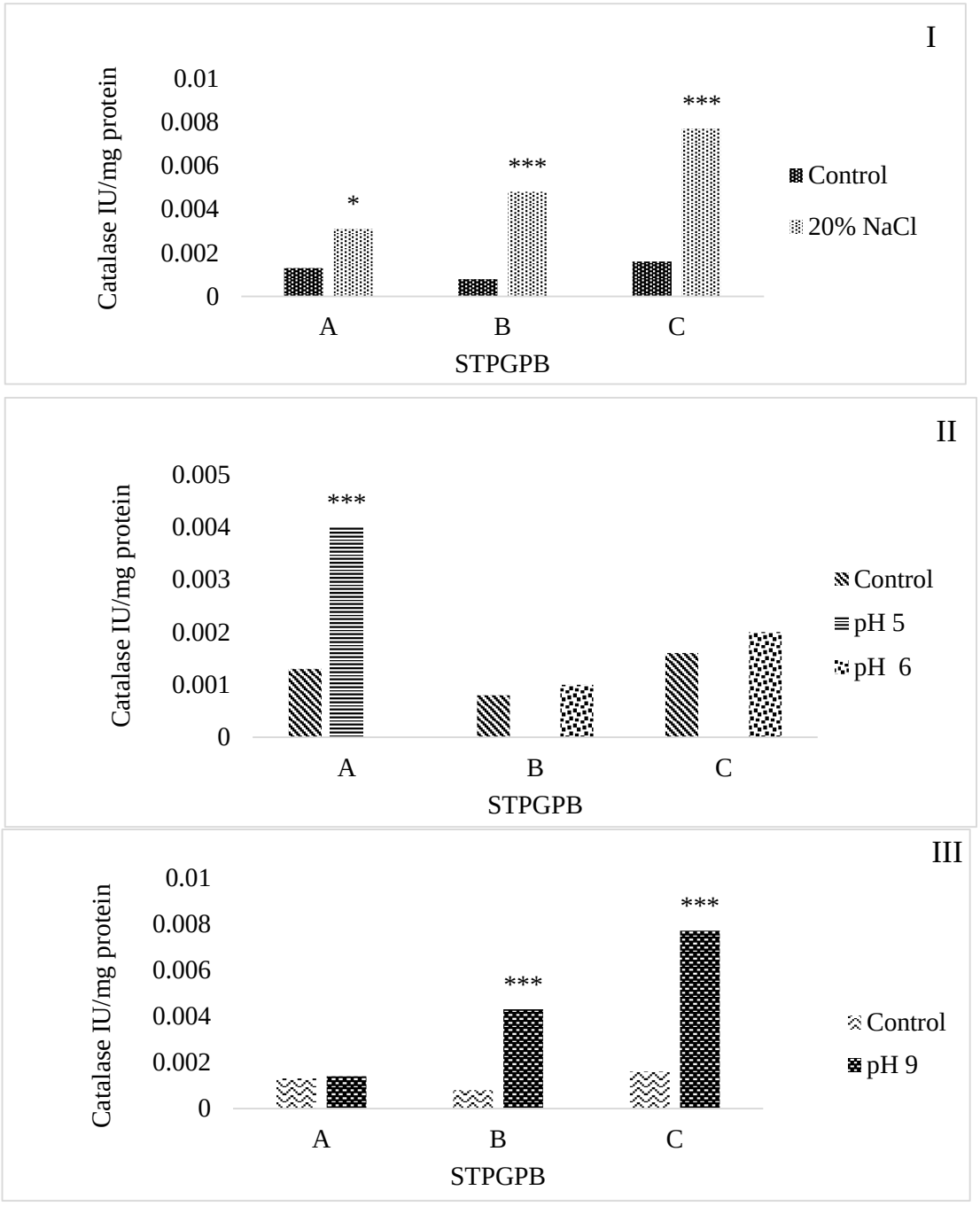


Figure 4.15. Comparison of CAT activity of STPGPB under abiotic stress

I= 20% NaCl, II = acidic pH (5 and 6), II= alkaline pH (9), A= *E. cloacae* (KP226569), B= *Enterobacter* sp. (KP226570), C= *A. nigricans* (KP966496), *p<0.05; **P<0.01; ***p<0.001

When subject to 20% NaCl *A. nigricans (*KP966496) and *Enterobacter* sp. (KP226570) expressed significant ($p<0.001$) high activity of CAT, whereas *E. cloacae* (KP226569) showed significant ($p<0.05$) CAT activity (**fig 4.15.I**).

Under abiotic stress of acidic pH contrary to results of 20 NaCl, only one organism *E. cloacae* (KP226569) showed significantly higher ($p<0.001$) CAT activity (**fig 4.15.II**). However, in other two organisms there was no significant increase in CAT activity under the stress of acidic pH (**fig 4.15.II**).

When subjected to alkaline pH 9 *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) expressed significantly high ($p<0.001$) CAT activity, whereas *E. cloacae* (KP226569) did not show significant CAT activity (**fig 4.15.III**).

4.6.1.3. Antioxidant enzyme GSH activity among STPGPB under abiotic

All the three organism studies for antioxidant enzyme GSH under abiotic stresses viz 20% salt, acidic pH (5-6) and alkaline pH and results are represented in **fig 4.16**. From the results it is clear that both *A. nigricans (*KP966496) and *Enterobacter* sp. (KP226570) expressed significantly high activity of GSH under all the three abiotic stresses (**fig 4.16**). However, in case of *E. cloacae* (KP226569) no significant response was observed under all the three abiotic stresses.

Many studies have reported that PGPB has increased the activity of antioxidant enzymes in plants under salt stress conditions (**Hediye Sekmen *et al*., 2007; M'Hamdi *et al*., 2009; Heidari *et al*., 2011, Heidari and Golpayegani , 2012; Gururani *et al*., 2013; Ahmad *et al*., 2015; Subramanian *et al*., 2015**). **Chatterjee and co-workers (2017)** reported that improved antioxidant activity in leaf tissues by inoculated salt resistance bacteria in plant. *B. aquimaris* DY-3 alleviated the salt stress in maize through the antioxidant enzymes (**Lia and Jiang, 2017**). It has been reported that plant associated microbes enhance antioxidant enzyme activities as systemic resistance tools against salt stress (**Abd Allah *et al*., 2015; Hashem *et al*., 2016**). The present study observed an increased activity of antioxidant enzymes such as SOD, CAT and GSH in PGPB which help to strengthen the antioxidant defence system.

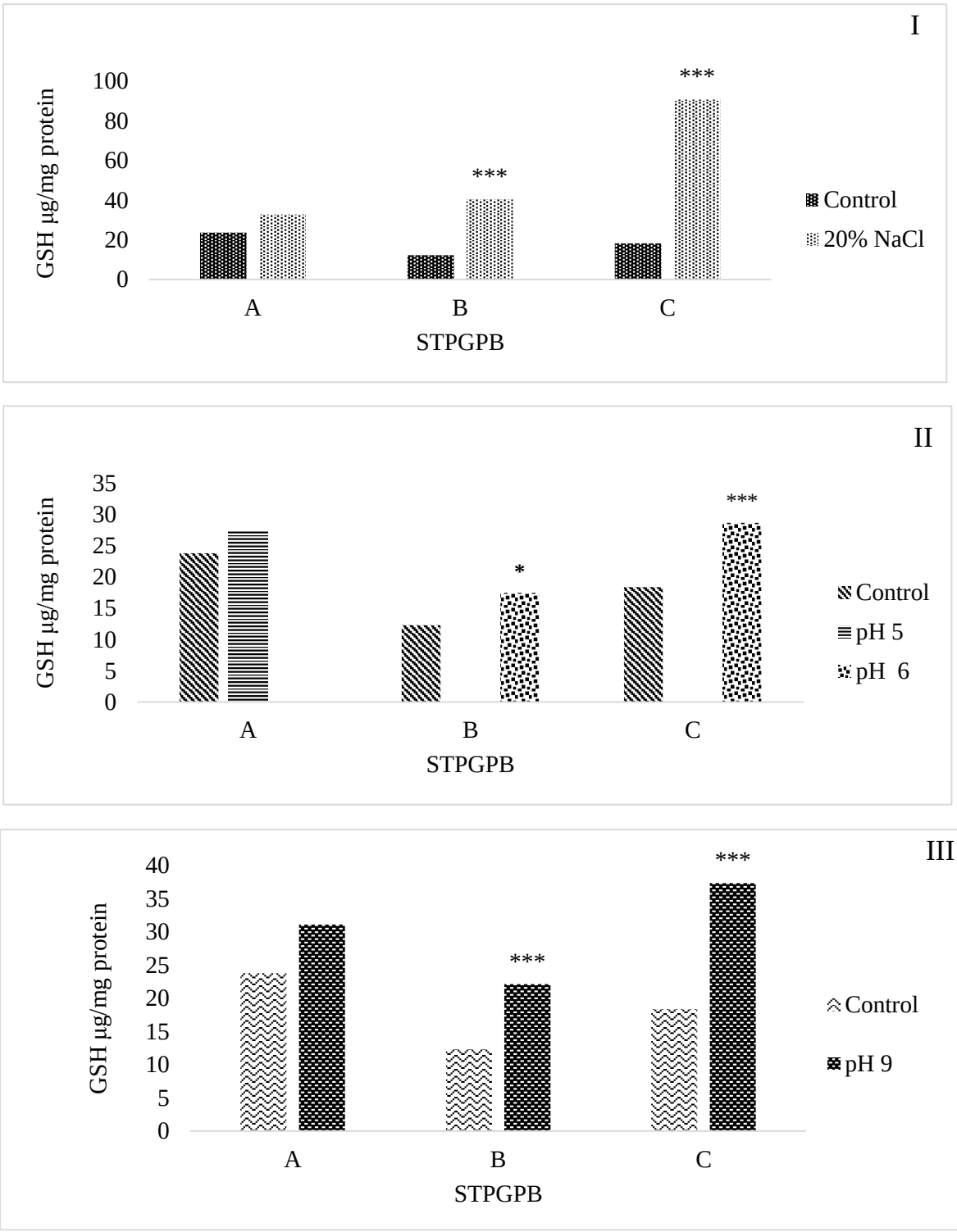


Figure 4.16. Comparison of GSH activity of STPGPB under abiotic stress

I= 20% NaCl, II = acidic pH (5 and 6), II= alkaline pH (9), A= *E. cloacae* (KP226569), B= *Enterobacter* sp. (KP226570), C= *A. nigricans* (KP966496), *p<0.05; **P<0.01; ***p<0.001

4.7. Inoculation effect of STPGPB on seed germination and growth parameters of various cereal crops

Three STPGPB viz. *E. cloacae* (KP226569), *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) were studied for their inoculation effect on seed germination and growth parameters of various crops *viz*. rice, maize, millets and wheat and results are given in **tables 4.15.- 4.18.**

4.7.1. Inoculation effect of STPGPB on seed germination and growth parameters of rice varieties

Nine varieties of rice were included to evaluate the inoculation effect of three STPGPB on seed germination and growth parameters (**Table 4.15**).

Enhanced seed germination and elongation of root and shoot were observed in rice verities of Sahbhagi and Leimaphou when inoculated with three STPGPB (**Table 4.15**). Elongation of root and shoot was noted in rice varieties of RC5 and CAUR-1. Similarly in rice varieties RC4, RC 10 and CAUR-3 only elongation of root was noted.

Table 4.15. Inoculation effect of STPGPB on seed germination and growth parameters of rice varieties at 9th DAS

Rice varieties	Percent germination				Elongation in cm							
					Root				Shoot			
	C	I	II	III	C	I	II	III	C	I	II	III
Sahbhagi	60	100*	100*	100*	6	13**	12**	14**	5	10*	13**	10*
Ginphou	80	100	100	100	3	3	5	5	4	6	5	7*
Leimaphou	60	100	100	80	2	4	5*	5*	2	3	4	6*
RC 4	100	80	100	100	6	8	9*	7	5	8	9	8
RC 5	100	100	100	100	2	5*	6**	6**	2	5*	9**	6**
RC 6	60	80	60	80	3	4	6	6	4	4	6	6
RCM-10	80	80	100	100	2	4	7**	4	6	8	9	9
CAUR-1	80	80	100	100	2	4	5*	5	2	4	5	6**
CAUR-3	100	100	100	100	5	9*	6	9*	5	7	9	9

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496) *p<0.05; **P<0.01; ***p<0.001

4.7.2. Inoculation effect of STPGPB on seed germination and growth parameters in maize varieties at 9th DAS

Five varieties of maize were included to see the inoculation effect of three STPGPB on seed germination and growth parameters (**Table 4.16**).

Table 4.16. Inoculation effect of STPGPB on seed germination and growth parameters in maize varieties at 9th DAS

Maize varieties	Percent germination				Elongation in cm							
					Root				Shoot			
	C	I	II	III	C	I	II	III	C	I	II	III
Kanchan	50	60	100*	50	4	8*	7	8	8	12	10	11
Azad Uttam	50	20	80*	60	4	9*	8*	10	4	12**	12**	9*
Saradmani	70	70	50	80	4	8*	9*	10	9	11	10	14
Navjyot	50	80	80	80	4	11*	10*	13	8	15*	13	15*
SHIATS MS -2	40	98*	80*	95*	4	13**	10*	12	9	14	14	12

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496),*p<0.05; **P<0.01; ***p<0.001

Significant increase in seed germination of maize varieties were noted in Kanchan, Azad Uttam and SHIATS MS-2, whereas marginal increase of seed germination was observed in maize variety Navjyot (**Table 4.16; Photo 1**). Significant increase in elongation of root length was observed in all five varieties of maize with inoculation of STPGPB. On the other hand increase in shoot length was noted in only two varieties of maize Azad Uttam and Navjyot.

4.7.3. Inoculation effect of STPGPB on seed germination and growth parameters in millets

Four millets varieties were included to see the inoculation effect of three STPGPB on seed germination and growth parameters (**Table 4.17**).

Table 4.17. Inoculation effect of STPGPB on seed germination and growth parameters in millets verities at 9th DAS

Millets variety	Percent germination				Elongation in cm							
					Root				Shoot			
	C	I	II	III	C	I	II	III	C	I	II	III
Normal sorghum SCV20	60	100*	100*	80	3	9**	18***	10**	4	12**	16***	13*
Sweet sorghum,CSH16	90	90	100	100	5	7	10*	7	9	12	12	10
Finger Millets CO14	50	100*	100*	80	2	12**	15***	10***	4	12**	10**	12**
Pearl Millets MH1642	90	90	90	90	5	9	10*	11*	4	8*	12**	7

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496) *p<0.05; **P<0.01; ***p<0.001

Significant increase in seed germination and growth parameters of finger millet CO14 was noted among all the millets under study, whereas marginal increase of seed germination was observed among other millets (**Table 4.17**). However, significant increase in elongation of root and shoot length was observed in normal sorghum SCV20 and pearl millet MH1642. Sweet sorghum CSH16 was least effected by the inoculation of STPGPB.

4.7.4. Inoculation effect of STPGPB on seed germination and growth parameters in wheat varieties at 9th DAS

Twenty eight varieties of wheat Four were included to see the inoculation effect of three STPGPB on seed germination and growth parameters (**Table 4.18**).

Table 4.18. Inoculation effect of STPGPB on seed germination and growth parameters in wheat varieties

Wheat variety	Percent germination				Elongation in cm							
					Root				Shoot			
	C	I	II	III	C	I	II	III	C	I	II	III
NIDW295XHI8636	80	80	88	80	6	10	8	9	8	15	10	9
HPD153XMASA499	92	95	80	80	4	9*	9*	7	10	12	16	13
SHIATS DW3	80	85	84	85	6	8	7	7	8	11	15	13
SHIATS DW5	48	50	52	45	6	9	12*	10	9	10	14	13
RAJ1535	40	45	40	40	4	7	11**	8*	7	10	13	9
SHIATS DW6	30	35	40	45	3	8*	7	6*	5	8	8	9
SHIATS DW2	36	40	40	45	8	12	9	10	8	10	14	10
SHIATS DW1	60	65	65	60	5	10	11	9	8	12	13	12
AVKD-3XRD1008	40	45	50	45	8	10	11	11	7	11	11	12
DBPY-02-03XMASA499	60	65	60	65	8	11	11	10	8	14	11	13
HI8653	90	90	98	95	4	10*	10*	8*	9	10	10	11
DBP-01-11	30	35	40	40	1	3*	3*	3*	4	7	9	9
RAJ6560	40	45	48	45	4	7	6	7	9	12	10.2	11
HD2009	50	50	52	55	5	8	7	8	10	14	17	10
NIDW-295	80	80	96	50	4	11*	10*	11*	8	11	11	10
PDW-300	80	80	96	80	4	7	10*	9*	6	10	12*	10
HPD-153 XMASA499	90	90	96	80	8	11	11	10	7	10	10	10
DBPY-02-03X RD1008	90	90	100	90	4	7	7	8*	7	10	9	10

3'AKDW-2997	90	90	96	90	4	8*	7	7	8	11	11	12
DBPY-02-03XMASA 499(White Awn)	90	90	100	90	6	10	12*	10	8	11	13	10
HPD153X HASA499 (White Awn)	90	90	100	90	7	10	14*	9	7	12	13	10
NIDW-299 XHI8636	90	90	100	90	7	11	14*	8	8	11	17	11
HPD153X RD1088	90	90	100	90	4	11*	13*	10*	9	12	14	10
AVKO-02 X HI8636 (White Awn)	90	90	92	95	8	13	10	10	8	10	10	10
AAI-W6	60	100	100	100	4	10*	8*	8*	7	15*	15*	11
K-9162	70	100	100	100	7	11	14*	9	6	15*	16*	12
Raj 3077	90	100	100	95	5	10*	16*	8	8	14	18*	10
HD-2786	70	100	100	100	4	10*	16***	8*	8	13	15*	10

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496) *p<0.05; **P<0.01; ***p<0.001

Surprisingly only marginal increase in seed germination and elongation of shoot were noted among wheat varieties inoculated with STPGPB (**Table 4.18**). However, significant increase in elongation of root length was observed among all varieties of wheat.

Several Researcher have been reported that PGPB significantly enhance seed germination and growth parameters with inoculation of PGPB in various cereal crops like rice (**Umashankari and Sekar, 2011; de Souza *et al.*, 2013; Midrarullah *et al.*, 2014 ; Sharma *et al.*, 2014)**, wheat (**Pereyra *et al.*, 2009 ; Mäder *et al.*, 2011; Gontia-Mishra *et al.*, 2017**), maize (**Piromyou *et al.*, 2011; Noumavo *et al.*, 2013).** PGPB are reported to influence the growth, yield, and nutrient uptake by an diverse array of mechanisms (**Saharan and Nehra, 2011**).

4.8. Inoculation effect of STPGPB on seed germination and growth parameters of cereals under salt stress

From the results obtained from above section few selected varieties of cereal crops were further included to see the inoculation effect of three STPGPB on seed germination and growth parameters under salt stress. The crops and their varieties included in this study are rice (Sahbhagi), maize (SHIATS MS-2 and Navjyot) and millets (normal sorghum SCV20 and finger millets CO14) and the results of these experiments are given in this section.

4.8.1. Inoculation effect of STPGPB on seed germination and growth parameters in rice var. Sahbhagi under salt stress

Rice var. Sahbhagi was included to see the inoculation effect of three STPGPB on seed germination and growth parameters under salt stress (**Fig 4.17**).

Inoculation with STPGPB significantly enhanced seed germination and growth parameter of rice var. Sahbhagi was noted in presence of 1-2 % salt, whereas marginal increase of seed germination was observed in rice variety Sahbhagi under 2% salt (**Fig 4.17**).

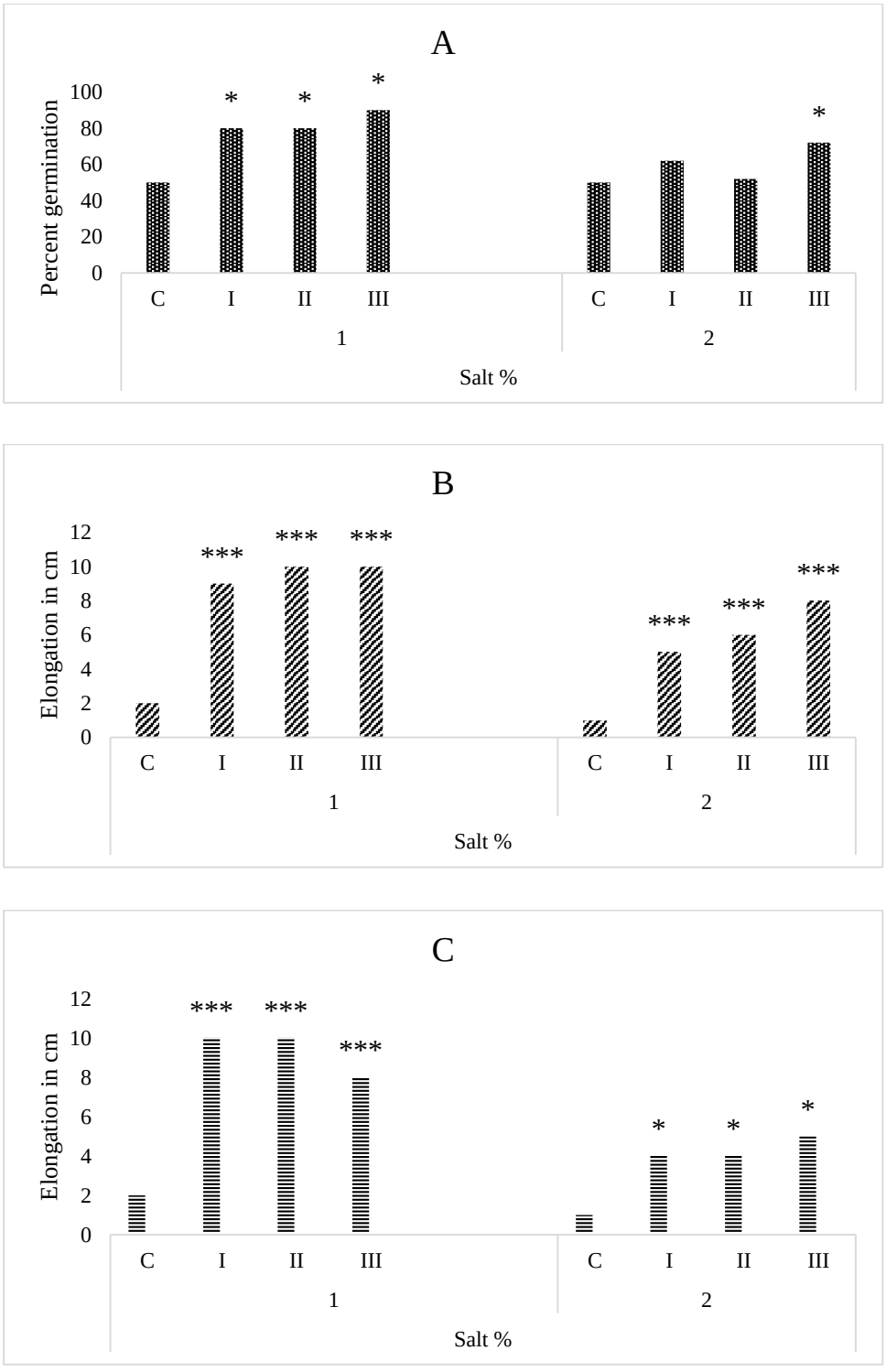


Figure 4.17. Inoculation effect of STPGPB on seed germination and growth parameters of rice var. Sahbhagi under salt stress

C= Control I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B=Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

4.8.2. Inoculation effect of STPGPB on seed germination and growth parameters in maize varieties under salt stress

Two maize varieties SHIATS MS-2 and Navjyot were included to see the inoculation effect of three STPGPB on seed germination and growth parameters under salt stress (**Fig 4.18 - 4.19; Photo 2-3**).

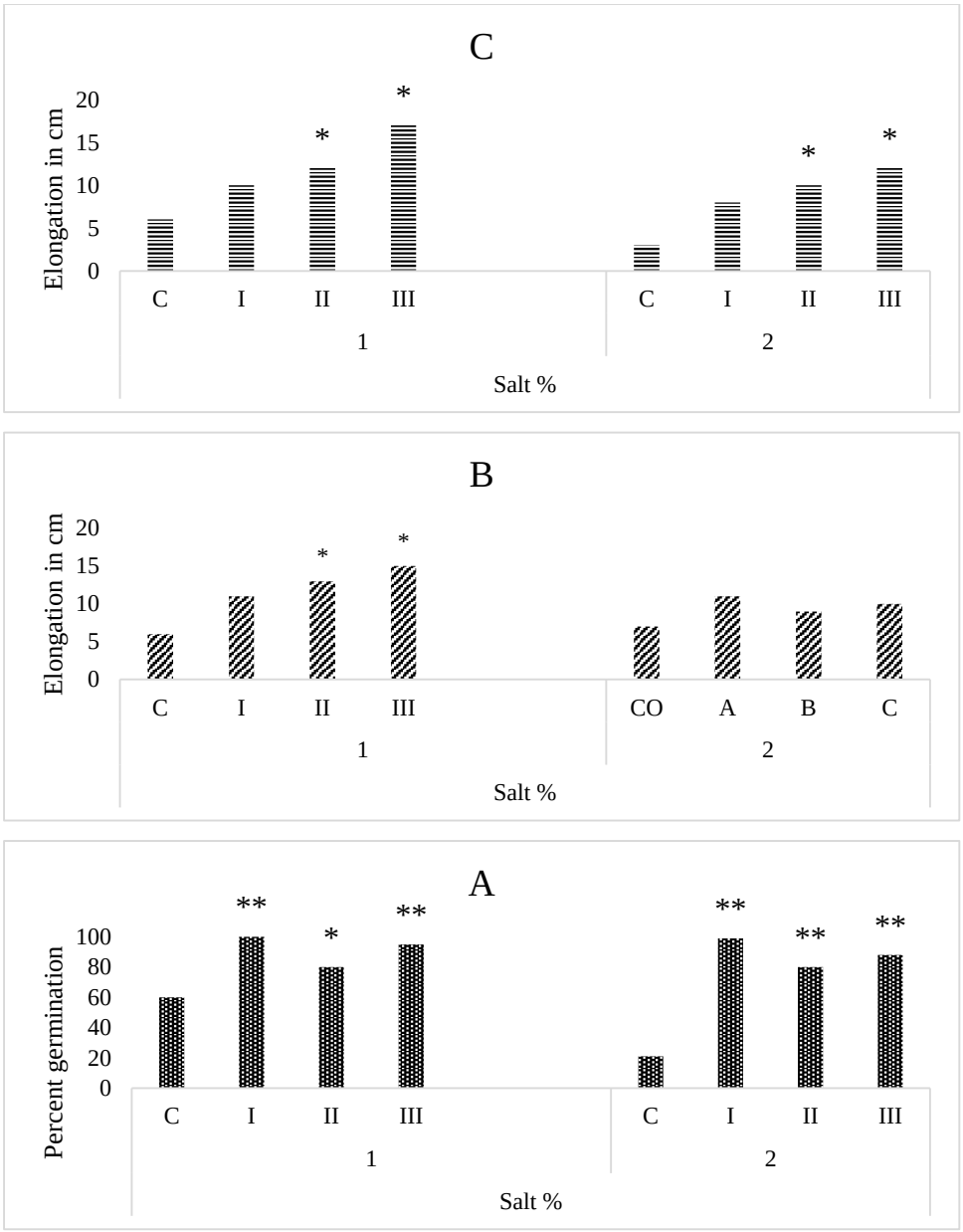


Figure 4.18. Inoculation effect of STPGPB on seed germination and growth parameters of maize var SHIATS MS-2 under salt stress

C= Control I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B=Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

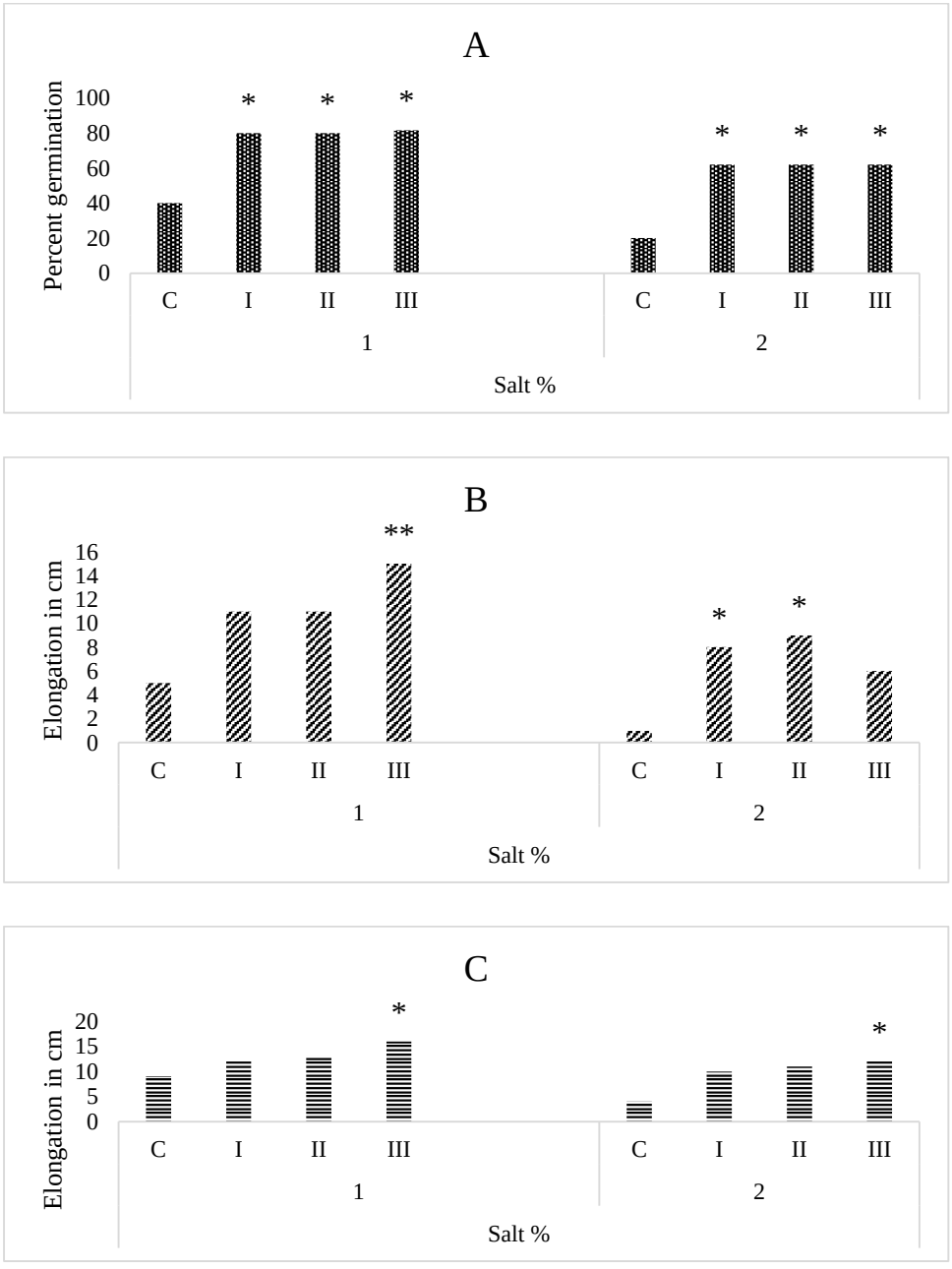


Figure 4.19. Inoculation effect of STPGPB on seed germination and growth parameters of maize var Navjyot under salt stress

C= Control I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B=Elongation of root, C= Elongation of shoot

Significant increase in seed germination and growth parameters of maize varieties SHIATS MS-2 and Navjyot were noted under salt stress (**Fig 4.18-4.19**), whereas marginal increase of elongation of root length under 2% salt was observed (**Fig 4.18**).

4.8.3. Inoculation effect of STPGPB on seed germination and growth parameters in millets under salt stress

Two millets viz. normal sorghum SCV20 and finger millet C014 were included to see the inoculation effect of three STPGPB on seed germination and growth parameters under salt stress (**Fig 4.20-21**).

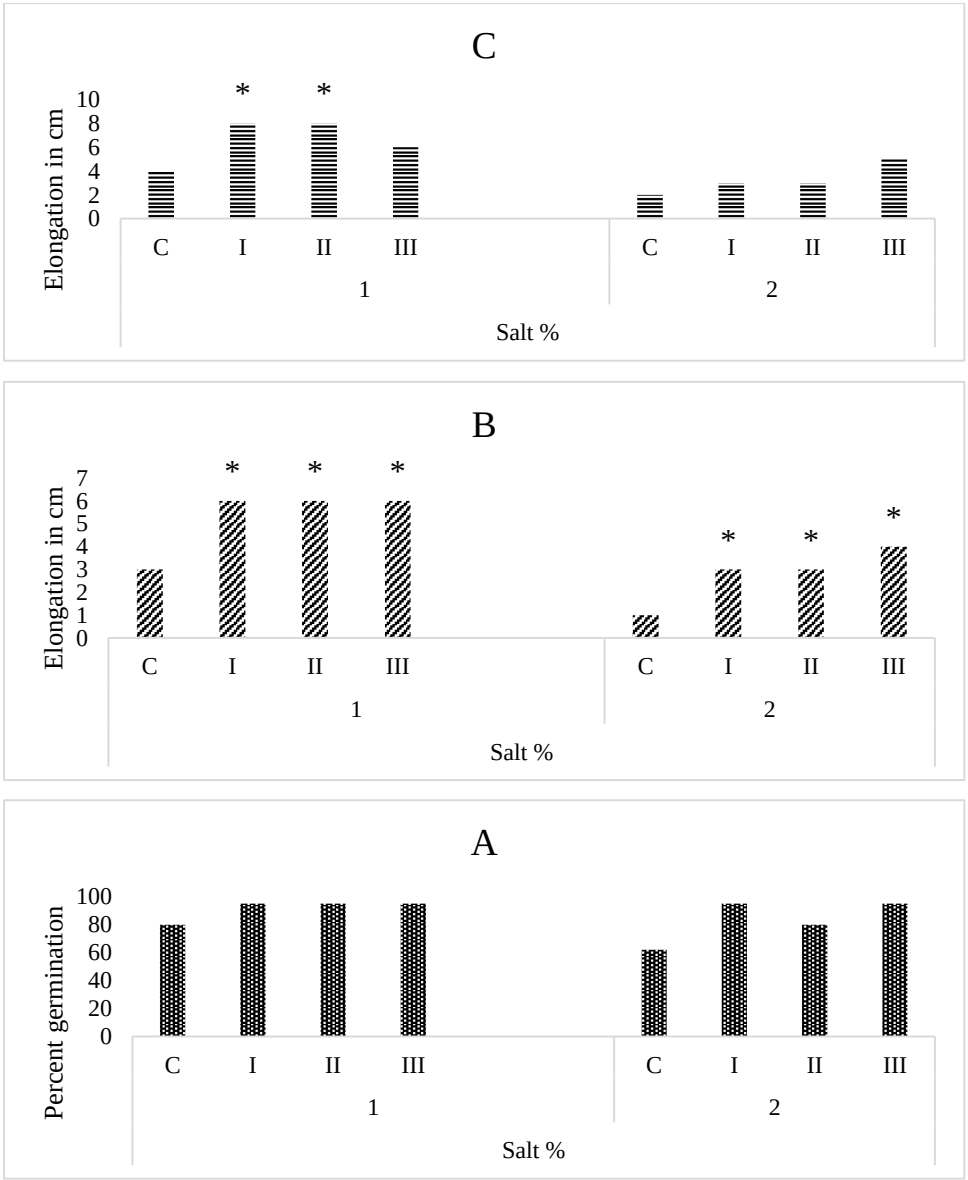


Figure 4.20. Inoculation effect of STPGPB on seed germination and growth parameters of millet viz. normal sorghum SCV20 under salt stress

C= Control I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496) A= Percent of Seed germination, B=Elongation of root, C= Elongation of shoot

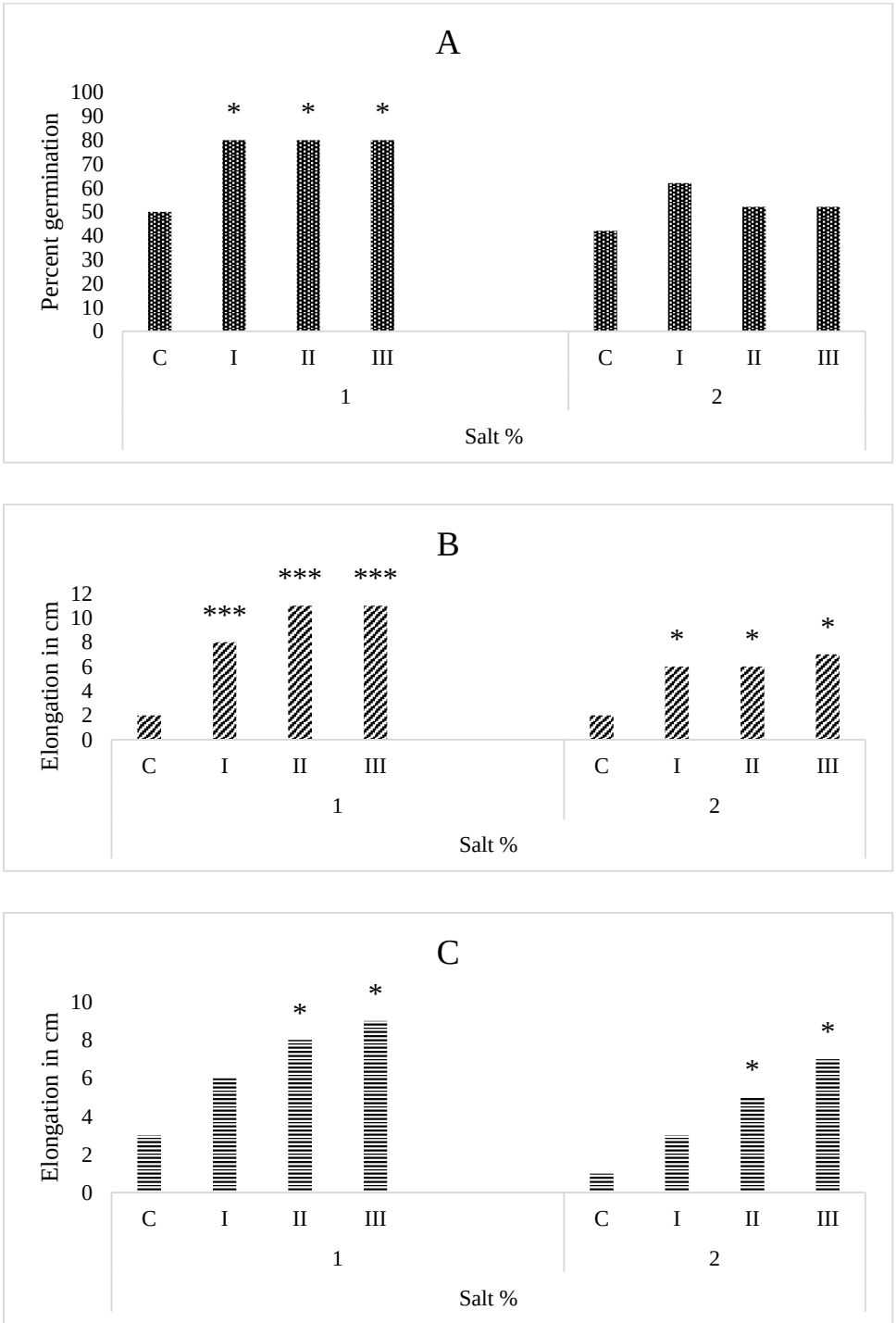


Figure 4.21. Inoculation effect of STPGPB on seed germination and growth parameters of finger millet CO14 under salt stress. C= Control I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed

germination, B=Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

In normal sorghum SCV20 all the three STPGPB did not effected seed germination under salt stress (**Fig. 4.20 A**) whereas in finger millet C014 significant increase in seed germination under 1% salt was observed (**Fig. 4.21 A**). Significant increase in elongation of root and shoot length of normal sorghum SCV20 under 1% salt was noted (**Fig 4.20**). In case of finger millet C014 inoculation with all the three STPGPB significantly increased elongation of root and shoot length under salt stress (**Fig. 4.21 B and C**).

There is clear evidence that a diverse group of root-associated microbes is essential for promoting plant adaptation to salinity (**Turner *et al.*, 2013; De-Zelicourt *et al.*, 2013; Tkacz and Poole, 2015**). In addition, several physiological, enzymatic and biochemical changes in plants after inoculation with PGPB have been suggested to help alleviate salt (**Han and Lee 2005a, b; Upadhyay *et al.*, 2011, 2012a**). There are a number of reports that demonstrate the efficacy of PGPB for promoting plant growth under salt stress conditions in different crops like rice (**Egamberdieva, 2011; Nautiyal *et al.*, 2013; Shah *et al.*, 2017**), wheat (**Upadhyay *et al.*, 2012; Rajput *et al.*, 2013*;* Singh *et al.*, 2017),** maize (**Akram *et al.*, 2016**; **Anzuay *et al.*, 2017). Shrivastava and Kumar 2015** reported that PGPB play a significant role in tolerance to saline conditions, genetic diversity, synthesis of compatible solutes, production of plant growth promoting hormones, bio-control potential, and their interaction with crop plants.

4.9. Inoculation effect of STPGPB on seed germination and growth parameters of cereals in presence of ammonium sulfate (substitute of ACC)

Three STPGPB viz. *E. cloacae* (KP226569), *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) were studied for their inoculation effect on seed germination and growth parameters of cereal crops *viz*. rice, maize and millets in presence of ammonium sulfate (substitute of ACC) and results are given in **fig 4.22 - 4.26.**

4.9.1. Inoculation effect of STPGPB on seed germination and growth parameters of rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC)

Inoculation effect of three STPGPB on seed germination and growth parameters in rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) is represented in **Fig. 4.22**.

Germination of seed of rice var. Sahbhagi was significantly ($p<0.05$) increased with inoculation of *E. cloacae* (KP226569) and *A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) as compared to control. Additionally, inoculation of three STPGPB significantly ($p< 0.001$) affected the increase of both root and shoot elongation in rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.22**).

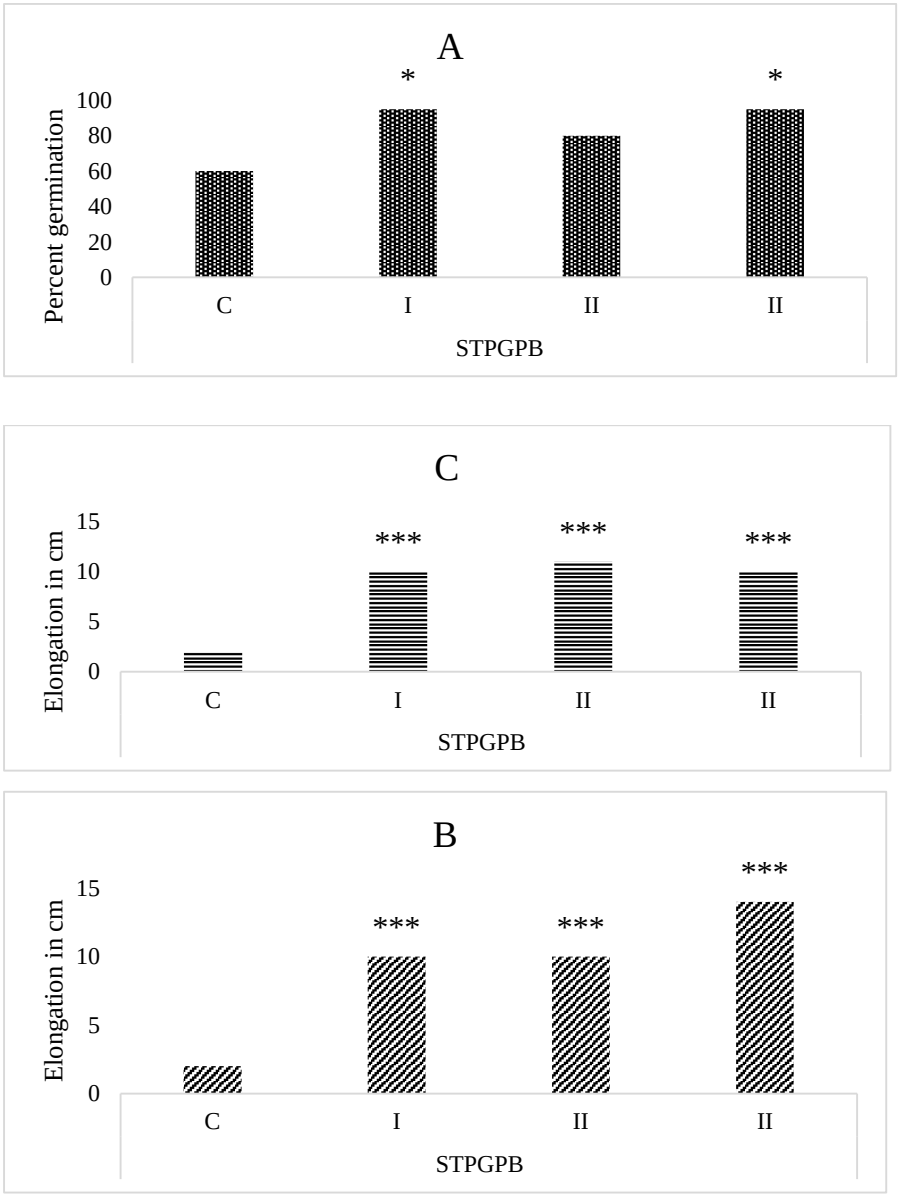


Figure 4.22. Inoculation effect of STPGPB on seed germination and growth parameters of rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC)

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

4.9.2. Inoculation effect of STPGPB on seed germination and growth parameters of maize varieties in presence of ammonium sulfate (substitute of ACC)

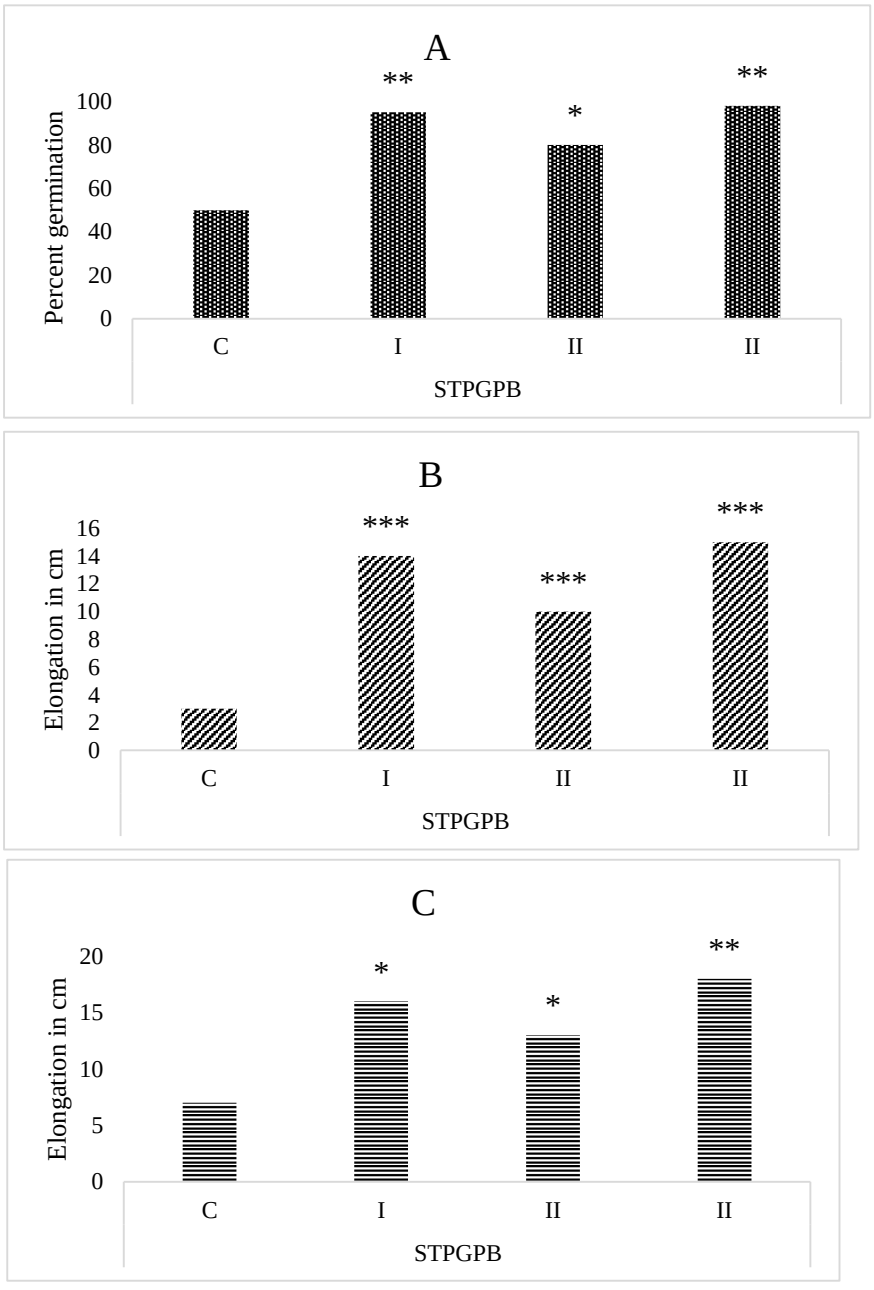


Figure 4.23. Inoculation effect of selected PGPB on seed germination and growth parameters of maize var SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC). C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot

Inoculation effect of three STPGPB on seed germination and growth parameters in maize var. SHIATS MS-2 and Navjyot in presence of ammonium sulfate (substitute of ACC) is represented in **Figs. 4.23 and 4.24; Photo 4-5**, respectively.

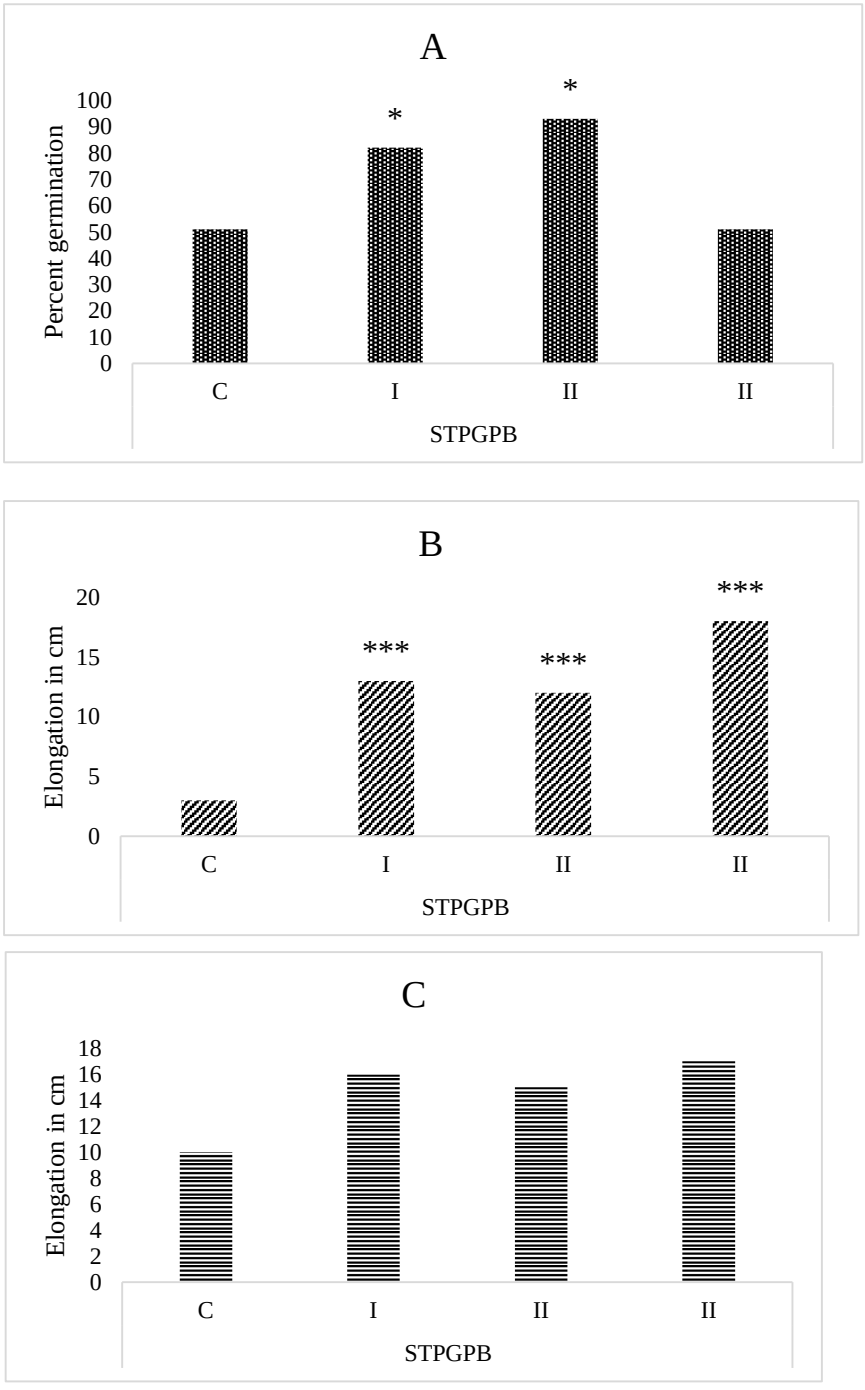


Figure 4.24. Inoculation effect of selected PGPB on seed germination and growth parameters of maize var Navjyot in presence of ammonium sulfate (substitute of ACC). C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

Inoculation effect of three STPGPB on seed germination and growth parameters in maize var. SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC) is represented in **Fig. 4.23**.

Inoculation of three STPGPB significantly ($p< 0.05$ to $p< 0.001$) affected the increase of seed germination and elongation of both root and shoot in maize var. SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.23**).

Inoculation effect of three STPGPB on seed germination and growth parameters in maize var. Navjyot in presence of ammonium sulfate (substitute of ACC) is represented in **Fig. 4.24.**

Germination of seed of maize var. Navjyot was significantly ($p<0.05$) increased with inoculation of *E. cloacae* (KP226569) and *Enterobacter* sp. (KP226570) in presence of ammonium sulfate (substitute of ACC) as compared to control (**Fig. 4.24 A**).

Inoculation of three STPGPB significantly ($p< 0.001$) affected the increase of elongation of root in maize var. Navjyot in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.24 B**). However, inoculation of three STPGPB did not affected the increase of shoot elongation in maize var. Navjyot in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.24 C**).

4.9.3. Inoculation effect of STPGPB on seed germination and growth parameters of millets in presence of ammonium sulfate (substitute of ACC)

Inoculation effect of three STPGPB on seed germination and growth parameters in millets viz. normal sorghum SCV 20 and finger millet C014 in presence of ammonium sulfate (substitute of ACC) is represented in **Figs. 4.25 and 4.26**, respectively.

Germination of seed of normal sorghum SCV 20 was significantly ($p<0.05$) increased with inoculation of *E. cloacae* (KP226569) and *Enterobacter* sp. (KP226570) in presence of ammonium sulfate (substitute of ACC) as compared to control (**Fig. 4.25 A**).

Inoculation of three STPGPB significantly ($p < 0.001$) affected the increase of elongation of root in normal sorghum SCV 20 in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.25 B**). However, significant ($p<0.05$) increase of shoot elongation with inoculation of *E. cloacae* (KP226569) and *A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) as compared to control was observed (**Fig. 4.25 C**).

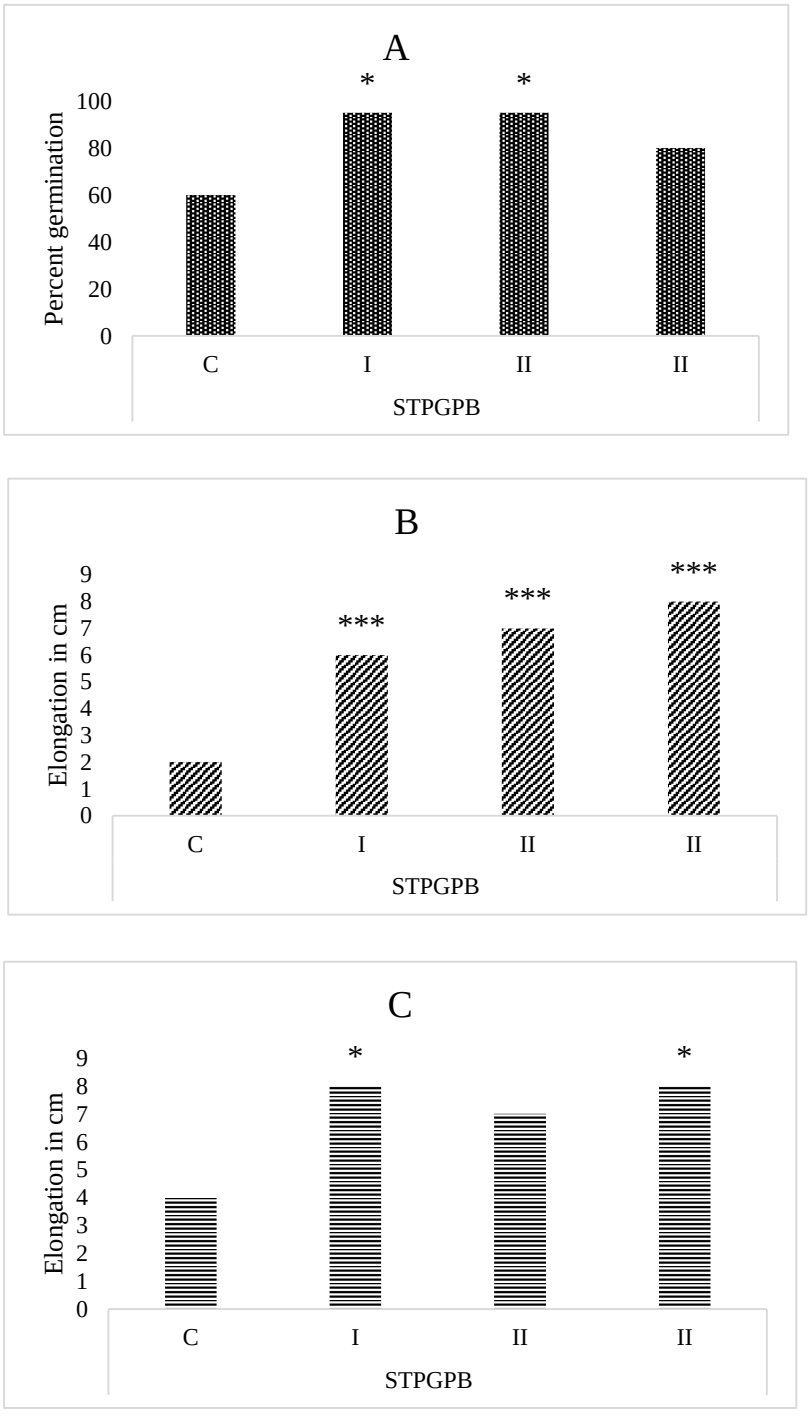


Figure 4.25. Inoculation effect of selected PGPB on seed germination and growth parameters of millet viz. normal sorghum SCV20 in presence of ammonium sulfate (substitute of ACC)

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans* (KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

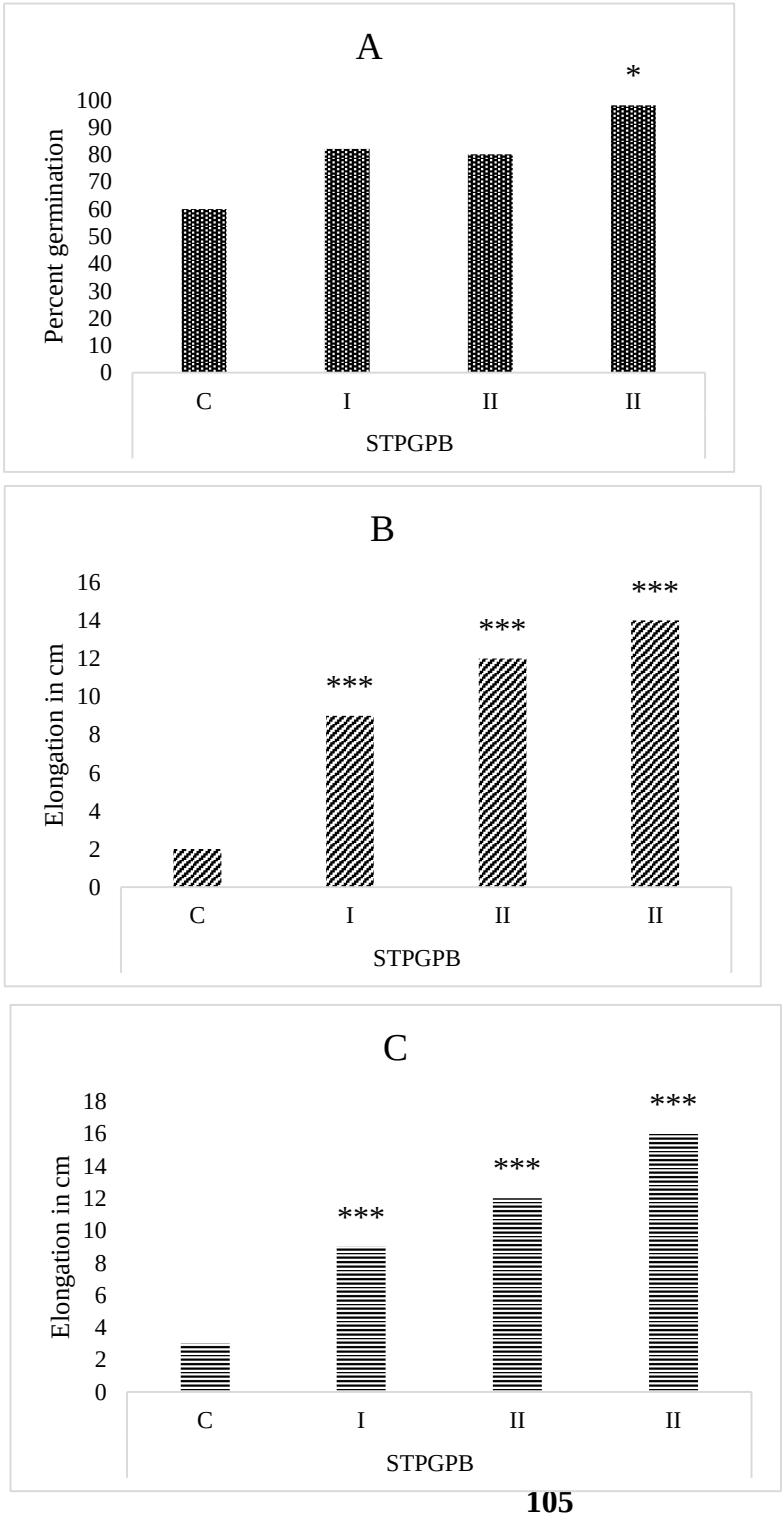


Figure 4.26. Inoculation effect of selected PGPB on seed germination and growth parameters of millet viz. finger millet CO14 in presence of ammonium sulfate (substitute of ACC) C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

Germination of seed of finger millet C014 was significantly ($p<0.05$) increased with inoculation of *A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) as compared to control (**Fig. 4.26 A**).

Inoculation of three STPGPB significantly ($p< 0.001$) affected the increase of elongation of both root and shoot in finger millet C014 in presence of ammonium sulfate (substitute of ACC) (**Fig. 4.26 B and C**).

4.10. Inoculation effect of STPGPB on seed germination and growth parameters of cereals in presence of ammonium sulfate (substitute of ACC) under salt stress

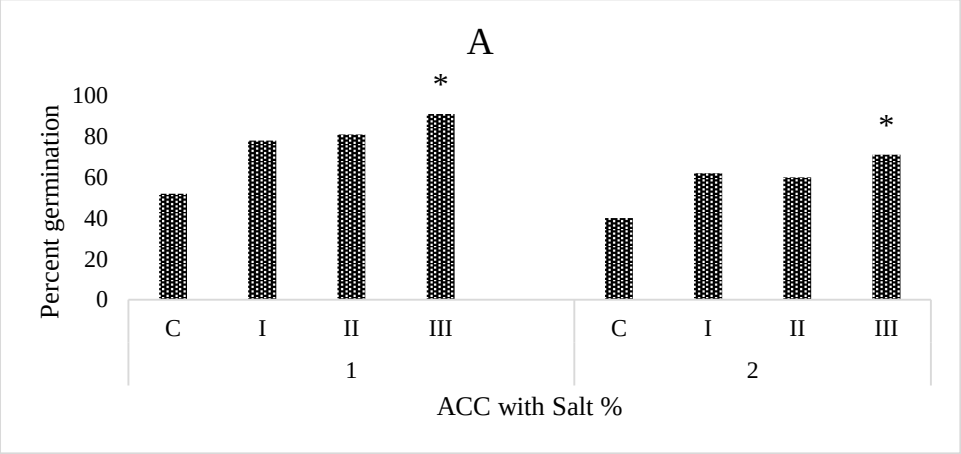
Three STPGPB viz. *E. cloacae* (KP226569), *Enterobacter* sp. (KP226570) and *A. nigricans (*KP966496) were studied for their inoculation effect on seed germination and growth parameters of cereal crops *viz*. rice, maize and millets in presence of ammonium sulfate (substitute of ACC) under salt stress and results are given in **fig 4.27 - 4.31**.

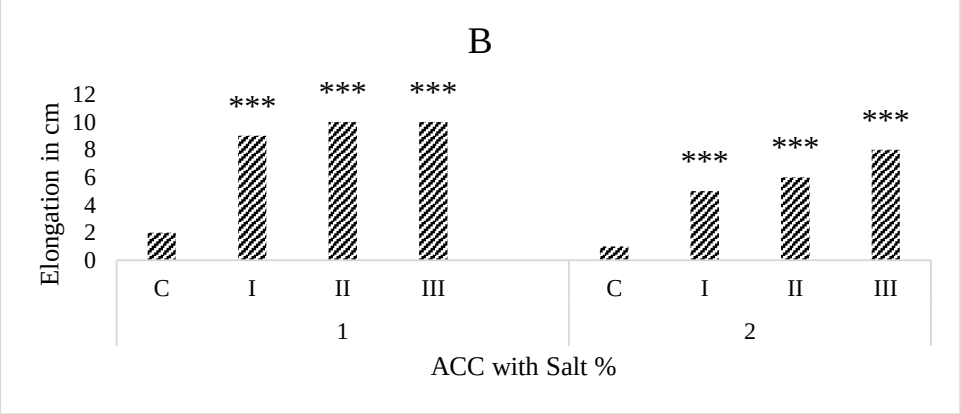
4.10.1. Inoculation effect of STPGPB on seed germination and growth parameters of rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) under salt stress

Inoculation effect of three STPGPB on seed germination and growth parameters of rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) under salt stress is represented in **Fig. 4.27**.

Germination of seed of rice var. Sahbhagi was significantly ($p<0.05$) increased with inoculation of *A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) under salt

stress as compared to control (**Fig. 4.27 A**). Inoculation of three STPGPB significantly ($p < 0.001$) affected the increase of elongation of both root and shoot in rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) under salt stress (**Fig. 4.27 B and C**).





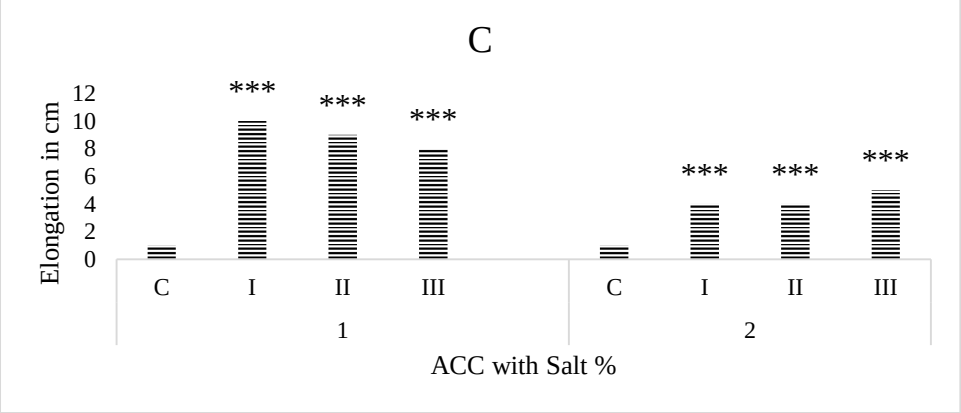


Figure 4.27. Inoculation effect of STPGPB on seed germination and growth parameters of rice var. Sahbhagi in presence of ammonium sulfate (substitute of ACC) under salt stress

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

4.10.2. Inoculation effect of STPGPB on seed germination and growth parameters of maize verities in presence of ammonium sulfate (substitute of ACC) under salt stress

Inoculation effect of three STPGPB on seed germination and growth parameters of maize var. SHIATS MS-2 and Navjyot in presence of ammonium sulfate (substitute of ACC) under salt stress is represented in **Fig. 4.28-4.29; Photo 6.**

Germination of seed of SHIATS MS-2 was significantly ($p<0.05$) increased with inoculation of three STPGPB in presence of ammonium sulfate (substitute of ACC) under salt stress as compared to control (**Fig. 4.28 A**).

Inoculation of three STPGPB significantly ($p< 0.05$) affected the increased elongation of root in SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC) under 1% salt stress, whereas under salt stress of 2% elongation of root was not observed (**Fig. 4.28 B**). However, significant ($p<0.05$) increase of shoot elongation with inoculation of *Enterobacter* sp. (KP226570) *and A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) under salt stress as compared to control was observed (**Fig. 4.28 C**).

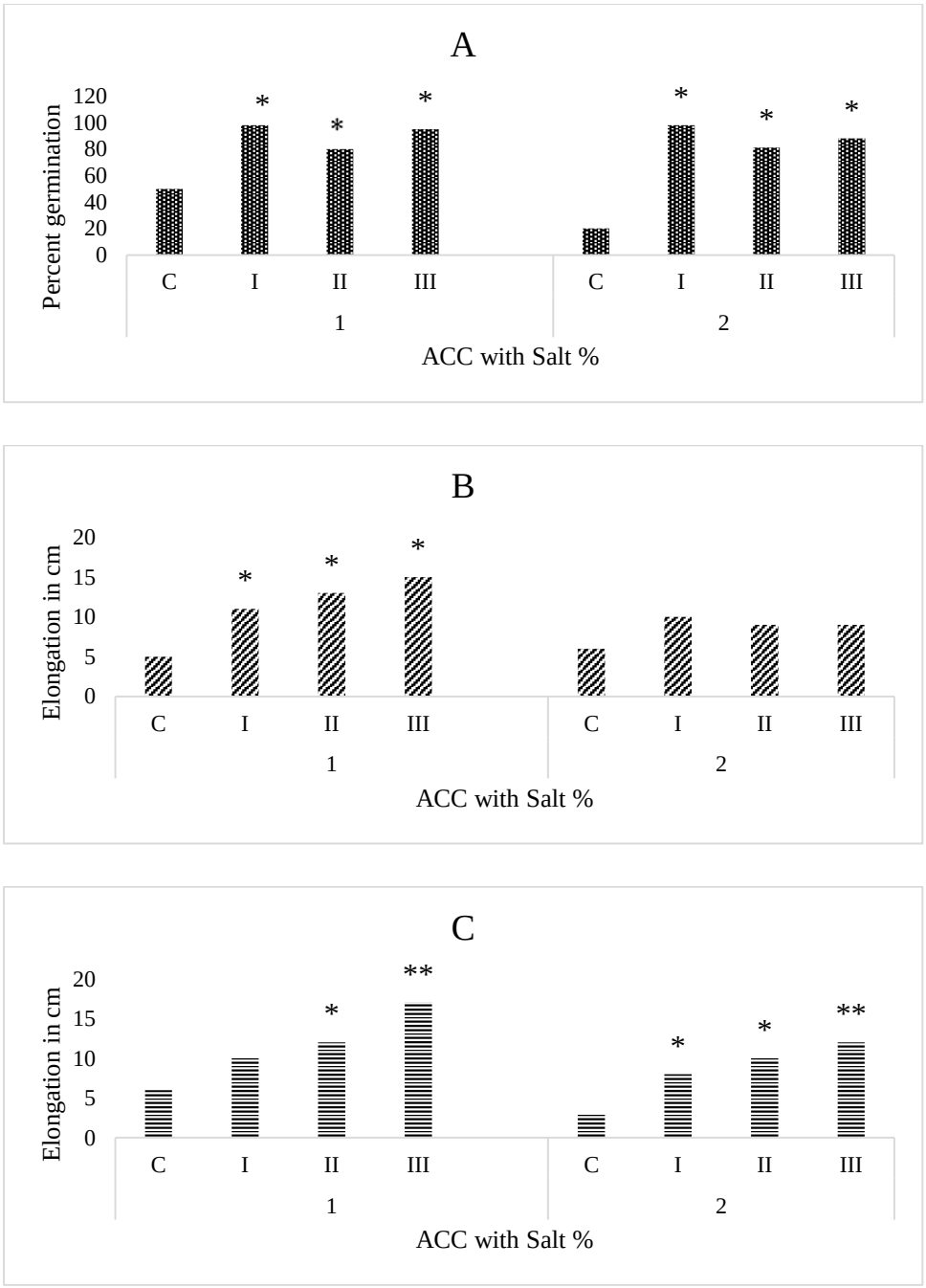


Figure 4.28. Inoculation effect of STPGPB on seed germination and growth parameters of maize var SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC) under salt stress. C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot

Inoculation of three STPGPB significantly ($p< 0.05$ to $p< 0.001$) affected the increase of seed germination and elongation of root in maize var. Navjyot in presence of ammonium sulfate (substitute of ACC) under salt stress (**Fig. 4.29 A- B**). However, significant ($p<0.05$) increase

of shoot elongation with inoculation of *A. nigricans (*KP966496) in presence of ammonium sulfate (substitute of ACC) under salt stress as compared to control was observed (**Fig. 4.29 C**).

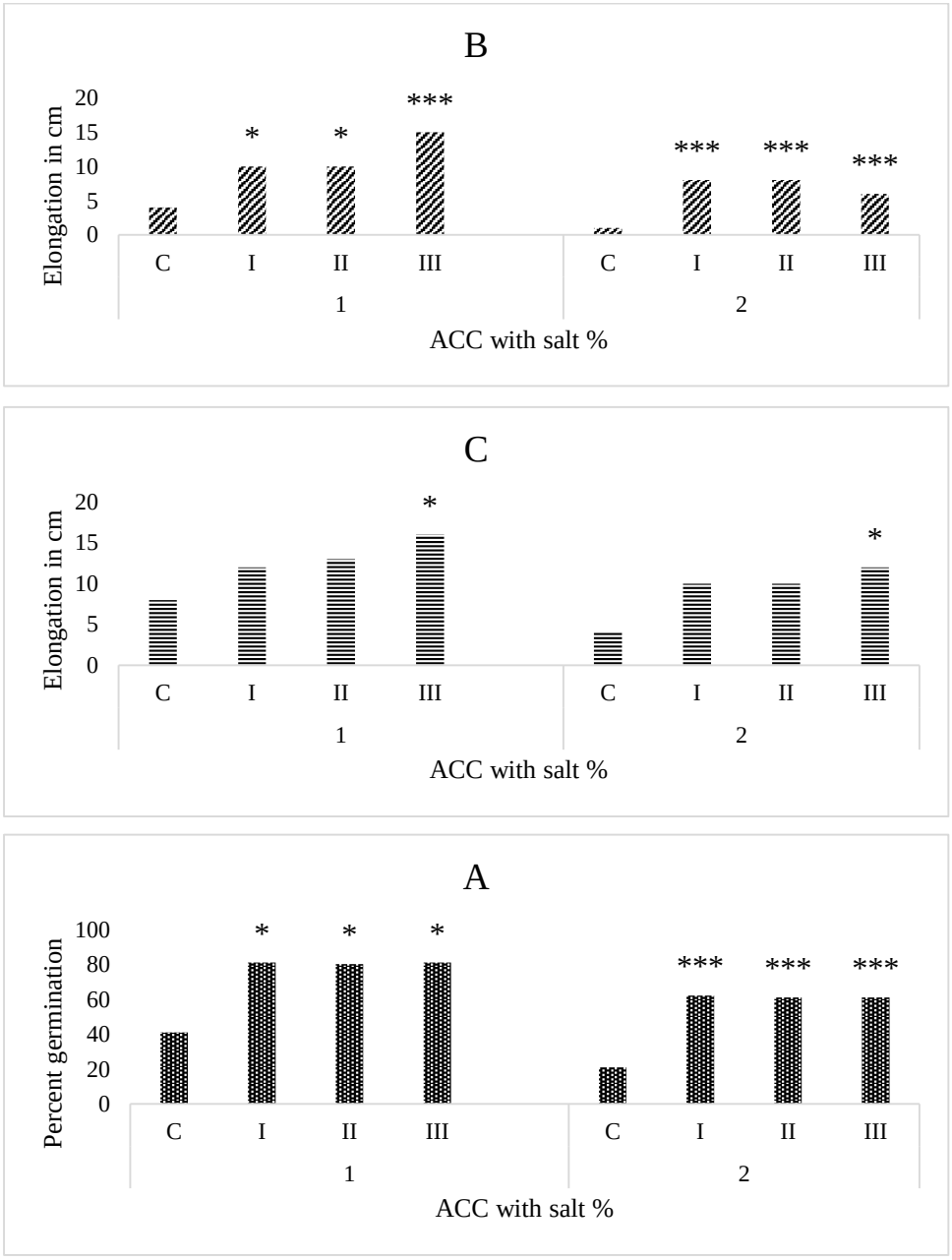


Figure 4.29. Inoculation effect of S PGPB on seed germination and growth parameters of maize var. Navjyot in presence of ammonium sulfate (substitute of ACC) under salt stress.

C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

4.10.3. Inoculation effect of STPGPB on seed germination and growth parameters of millets in presence of ammonium sulfate (substitute of ACC) under salt stress

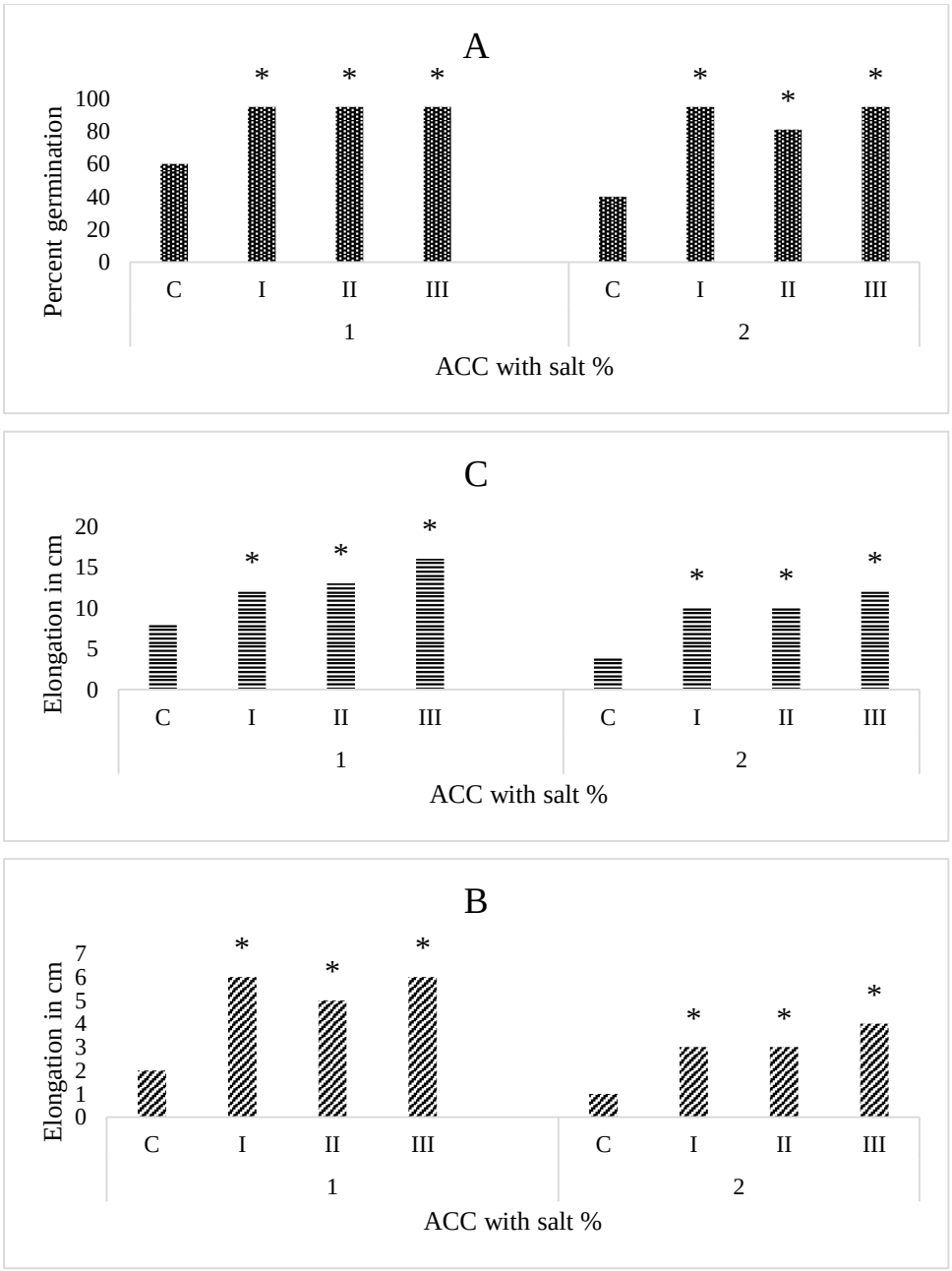


Figure 4.30. Inoculation effect of selected PGPB on seed germination and growth parameters of millet viz. normal sorghum SCV20 in presence of ammonium sulfate (substitute of ACC) under salt stress. C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot

Inoculation effect of three STPGPB on seed germination and growth parameters in millets viz. normal sorghum SCV 20 and finger millet C014 in presence of ammonium sulfate (substitute of ACC) under salt stress is represented in **Figs. 4.30 and 4.31**, respectively.

Inoculation of three STPGPB significantly ($p< 0.05$) affected the increase of seed germination and both elongation of root and shoot of in millets viz. normal sorghum SCV20 in presence of ammonium sulfate (substitute of ACC) under salt stress (**Fig. 4.30**).

Germination of seed of millets viz. finger millet C014 was significantly ($p<0.05$) increased with inoculation of three STPGPB in presence of ammonium sulfate (substitute of ACC) under 1% salt stress as compared to control (**Fig. 4.31 A**). However, under salt stress of 2% germination seeds was not detected.

Inoculation of three STPGPB significantly ($p<0.05$ to $p<0.001$) affected the increase elongation of root and shoot in millets viz. finger millet C014in presence of ammonium sulfate (substitute of ACC) under salt stress (**Fig. 4.31 B-C**).

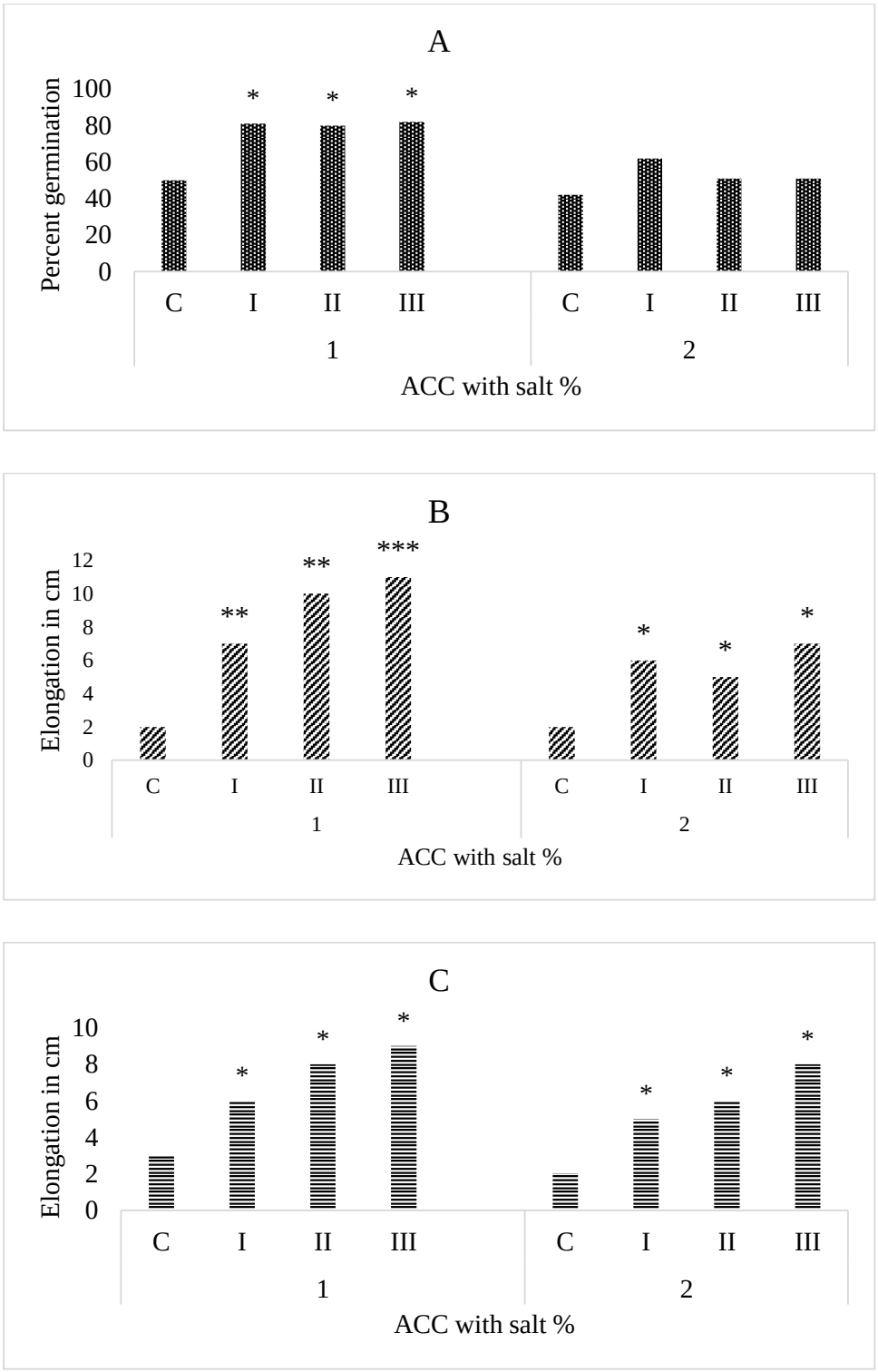


Figure 4.31. Inoculation effect of selected PGPB on seed germination and growth parameters of millet viz. finger millet CO14 in presence of ammonium sulfate (substitute of ACC) under salt stress C= Control, I= *E. cloacae* (KP226569), II= *Enterobacter* sp. (KP226570), III= *A. nigricans (*KP966496), A= Percent of Seed germination, B= Elongation of root, C= Elongation of shoot, *p<0.05; **P<0.01; ***p<0.001

PGPB that produces ACC deaminase have been shown to increase root elongation, improve seedling survival, and enhance stress tolerance (**Glick, 2005; Glick et al., 2007; Glick, 2014**). It has been proposed that root elongation results when ACC deaminase activity produced by such strains breaks down ethylene, which inhibits root elongation. PGPB with ACC deaminase is recognized as important for plants to adapt to abiotic and biotic stresses (**Saravanakumar and Samiyappan, 2007**). InIndeed bacteria can protect plants from the effects of salt via production of IAA, ACC deaminase, volatile organic compounds and exopolysaccharides **(Kim *et al.*, 2014; Forni *et al.*, 2017**). With respect to soil salinity, bacteria carrying traits for phosphate solubilization, synthesis of siderophores, exopolysaccharides and indole acetic acid (IAA) and with high ACC-deaminase enzyme activity were shown to be effective PGPB (**Nadeem *et al.*, 2010; Upadhyay *et al.*, 2011; Glick, 2014**).

PGPB that produce ACC deaminase have been shown to increase root elongation, improve seedling survival, and enhance stress tolerance. It has been proposed that root elongation results when ACC deaminase activity produced by such strains breaks down ethylene, which inhibits root elongation. Inhibition of plant growth by salinity is due to the toxic effects of NaCl on the root system to control entry of ions to the shoot and to slowing down water uptake of plants (**Glick, 2005; Glick *et al.*, 2007; Glick, 2014**).

In contrast, when the seeds were inoculated with the PGPB suspension, seed germination was reduced at a lower rate despite increasing salinity. This may indicate that the ACC deaminase-containing PGPB are able to ameliorate the effect of NaCl on growth medium. The PGPB got attached to the seed surface and synthesized phytohormones in response to amino acids produced by the seeds perhaps alleviating the salinity stress (**Patten and Glick, 2002**).

ACCD producing PGPB have been reported potential to promote seed germination and growth parameters in many crops like rice (**Bal *et al.*, 2013; Etesami *et al.*, 2014),** wheat **(Zahir et al. 2009; Nadeem et al. 2010; Ramadoss *et al.*, 2013; Singh and Jha, 2016**), maize (**Nadeem et al. 2009; Zafar-ul-Hye *et al.*, 2014**). These result conformant that the improved screening method shows real potential of ACCD producing bacteria.

4.11. Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of cereals under field condition

On the above experiment *E. cloacae* (KP226569) was selected for their inoculation effect on morphological and yield parameters of cereal crops *viz*. millets (finger millets CO14), wheat (var.AAI-W6) and maize (var. SHIATS MS-2) under field condition and results are given in **tables 4.19-4.21**.

4.11.1. Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of finger millet (*Elusine coracana* (L.) Gaertn.) CO14 under field condition

Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of finger millets CO 14 under field condition is represented in **table 4.19**.

Marginal increase in morphological and yield parameters of finger millet C0 14 was observed with inoculation of *E. cloacae* (KP226569) (**Table 4.19**). However, significant increase morphological and yield parameters of finger millet C014 was observed with inoculation of *E. cloacae* (KP226569) and NPK.

Table 4.19. Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of finger millet (*Elusine coracana* (L.) Gaertn.) CO14 under field condition

Agronomic traits	**Control**	**Std. NPK**	***E. cloacae* (KP226569)**	***E. cloacae* (KP226569) with NPK**
Plant height (cm)	70	85	90	95
Flag leaf sheath length (cm)	18	20	20	22
Flag leaf sheath width (cm)	8	10	10	15
Leaf number	10	12	16	16
Days to flowering	60	72	76	80
Days to maturity	3	4	6	4
Leaf Chlorophyll (mg/g)	2	2	3	3
Relative water content (%)	90	95	95	105
Number of tillers / plant	3	4	4	6
Number of productive tillers / plant	2	4	4	5
Number of fingers / head	3	6	6	9
Length of fingers (cm)	6	8	8	10
Finger width (cm)	1	2	2	3
Length of root (cm)	13	20	20	25
Grain yield per plant (g)	15	18	19	24
Thousand grain weight (g)	1	2	2	4
Harvest Index	55	63	75	80

4.11.2. Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of wheat (*Triticum aestivum* L.) var. AAI-W6 under field condition

Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of wheat var. AAI-W6 under field condition is represented in **table 4.20 and photo 7**.

Marginal increase in morphological and yield parameters of wheat var. AAI-W6 was observed with inoculation of *E. cloacae* (KP226569) (**Table 4.20**). However, significant increase morphological and yield parameters of wheat var. AAI-W6 was observed with inoculation of *E. cloacae* (KP226569) and NPK.

Table 4.20. Inoculation effect of *E. cloacae* (KP226569) on morphological, biochemical and yield parameters of wheat (*Triticum aestivum* L.) var. AAI-W6 under field condition

Agronomic traits	**Control**	**Std. NPK**	***E. cloacae* (KP226569)**	***E.cloacae* (KP226569) with NPK**
Plant height(cm)	42	50	52	56
Flag leaf width (cm)	1	2	2	2
Flag leaf length (cm)	12	15	17	20
Average no of Tiller/Plant	2	2	3	3
Length of spike (cm)	8	9	10	11
Weight of spike (g)	2	3	3	4
Fresh weight of plant (g)	11	13	15	17
Dry weight of plant (g)	2	3	5	7
Total Chlorophyll (mg/g)	2	3	4	5
Relative water content (%)	40	47	55	65
Seed Protein (%)	5	7	9	12
Total carbohydrate (g)	58	62	65	68
Grain yield per plant (g)	12	15	16	20
Number of grain per spike.	20	22	26	34
Average 1000 seed weigh (g)	20	22	27	38
Harvest index (%)	20	25	32	35

4.11.3. Inoculation effect of STPGPB on morphological and yield parameters of maize (*Zea mays* L.) var. SHIATS MS-2 under field condition

Inoculation effect of *E. cloacae* (KP226569) on morphological and yield parameters of maize var. SHIATS MS-2 under field condition is represented in **table 4.21and photo 8**.

Marginal increase in morphological and yield parameters of maize var. SHIATS MS-2 was observed with inoculation of *E. cloacae* (KP226569) (**Table 4.21**). However, significant increase morphological and yield parameters of maize var. SHIATS MS-2 was observed with inoculation of *E. cloacae* (KP226569) and NPK.

Table 4.21. Inoculation effect of STPGPB on morphological and yield parameters of maize (*Zea mays* L.) var. SHIATS MS-2 under field condition

Agronomic traits	**Control**	**Std. NPK**	***E. cloacae* (KP226569)**	***E. cloacae* (KP226569) with NPK**
Plant height(cm)	190	200	201	215
Flag leaf width (cm)	3	7	9	7
Flag leaf length (cm)	32	42	43	52
Length of cob (cm)	7	8	10	10
Weight of cob (g)	149	160	162	168
Fresh weight of plant (g)	360	370	378	385
Dry weight of plant (g)	205	270	275	280
Total Chlorophyll (mg/g)	20	28	30	30
Relative water content (%)	68	75	80	85
Number of grain per cobs	360	370	378	390
Protein (%) in seed	7	10	10	14
Total carbohydrate (%)	48	57	60	65
Grain yield per plant (g)	119	128	130	140
Average 1000 seed (g) weight	310	390	410	430
Harvest index (%)	132	142	145	150

Plant growth promoting effects of PGPB strains in different crops were clearly demonstrated (**Wu *et al.*, 2005**). Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease (**Lugtenberg *et al.*, 2002**). PGPB are naturally occurring soil bacteria that colonize plant roots and promote plant growth. PGPB treatment as an eco-friendly, cost effective, simple, easy deployable and potential tool for productivity enhancement in millet (**Saxena *et al.*, 2013).**PGPB as an environmentally sustainable approach to increase crop production and health. With the advancement in our understanding of their diversity, colonization ability, mechanisms of action, formulation, and application, the PGPB can develop as reliable components in the management of sustainable agricultural systems **(Saxena *et al.*, 2013**). In pot and field experiment, it was observed that inoculation with PGPB strains significantly promoted growth of seedling maize under different soil conditions. In general, inoculation resulted in early seedling growth and development (**Dobbelaere *et al.*, 2002**). Inoculation of maize seeds with PGPB significantly increased plant height, 100 seed weight, number of seed per ear and leaf area .The results also showed significant increase in ear and shoot dry weight of maize **(Gholami *et al.*, 2008).**A strong increase in total plant and grain dry weight was obtained when maize plants were inoculated with PGPB (**Riggs et al. 2001**) **Swędrzyńska, and Sawicka (2000)** found that inoculation of maize crops with an active strain of PGPB has a beneficial effect on maize vigor and yield. **Dobbelaere *et al.*, 2001** who assessed the inoculation effect of PGPB Azospirillum brasilense on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and also increase in dry weight of both the root system and the upper plant parts. Similarly, promotion in growth parameters and yields of various crop plants in response to inoculation with PGPB were reported by other workers (**Kozdroja *et al.*, 2004; Shaharoona *et al.*, 2006; Grave *et al.*, 2007;**). PGRB inoculation could significantly increase the growth in terms of height; number of leaf/plant; length and breadth of leaf; and fresh and dry weight/plant of rice plant (**Rodrigues *et al.*, 2008**; **Hossain, *et al.*, 2015**). Inoculation of seed with PGPB resulted higher plant height at time of harvest, yield attributing characters and yield of millet (**Abdullahi *et al.*, 2014**; **Patel *et al.*, 2014**). In inoculation of PGPB strains increased all parameters determined in field experiment. The positive effects of PGPBs on the yield and growth of crops such as wheat (**Kader, et al 2002; Ozturk *et al.*, 2003; Khalid *et al.*, 2004; Salantur *et al.*, 2006 ; Ahmed *et al.*, 2011**; **Kandil *et al.*, 2011**; **Abd El-Lattief, 2014**; **Yousefi and Barzegar, 2014)**. Grain yield of maize increased with inoculation by PGPB (**Gholami *et al.*, 2009**; **Hajnal-Jafari *et al.*, 2012**; **Naseri *et al.*, 2013**; **Baral and Adhikari, 2013**).

SUMMARY

The present study entitle "**Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylatedeaminase (ACC deaminase) in improving growth and yield of cereal crops under salinity stress"** has drawn to following summary:

- The soil samples were collected from SMOF, Allahabad.

- A total 650 bacterial isolates from SMOF were screed for MPGP traits and tolerant to trace elements.

- Twenty nine (29) potential PGPB out of six hundred fifty (650) isolates were identified by partial sequence analysis of 16S rRNA gene. On the basis of 16S rRNA sequence these potential PGPB were identified different groups of bacteria viz., *Enterobacter* sp. (15), *Pseudomonas* sp. (04), *Rhizobium* sp (03), *Azotobacter* sp. (02), and other sp. *Erwinia* sp.(01), *Inqilinus* sp.(01), *Escherichia hermannii (01)* and *Bacillus subtillis* (02) respectively .The obtained sequence was submitted to the NCBI gene bank and got different Accession no.

- On the basis of tolerance to high salt (20%), three STPGPB were selected for further detailed studies viz *E. cloacae* (KP226569) (PR4), *Enterobacter* sp. (KP226570) (PR14) and *A. nigricans (*KP966496) (PR19).

- It was observed that three STPGPB were highly tolerant for Al, Zn and Mo and least tolerance was observed against Hg. Similarly these STPGPB were resistance to MAR. It was also observed that STPGPB were highly significant ($p<0.001$) of antioxidant enzyme activity.

- Three STPGPB were used for improvement of seed germination and growth parameters of cereal crops viz. rice, maize, wheat and millets at laboratory and field under various condition.

- Three STPGPB showed significantly enhancement in seed germination (%) and growth parameters of cereal crops viz rice, maize, millets and wheat.

- Enhanced seed germination and elongation of root and shoot were observed in cereals crops when inoculated with three STPGPB under salt stress.

- Significant increase in seed germination (%) and both elongation of root and shoot was observed in rice, maize and millets with inoculation of three STPGPB in presence of ammonium sulfate (substitute of ACC).

- All cereal crops showed significant enhance of percent seed germination and growth parameters in presence of ammonium sulfate (substitute of ACC) under salt stress by three STPGPB.

- In field condition, significantly enhanced in morphological and yield parameters of finger, millets wheat and Maize by *E. cloacae* (KP226569).

CONCLUSION

The present study entitle "**Evaluation of performance of plant growth promoting bacteria (PGPB) containing 1-aminocyclopropane- 1-carboxylatedeaminase (ACC deaminase) in improving growth and yield of cereal crops under salinity stress"** has drawn to the following conclusion:

- The present study we observed rich bacterial diversity in soil from SMOF both in terms of their types and functional (PGP) traits. 650 bacterial isolates were predominantly positive to production of ammonia, IAA, catalase, ACCD and siderophore. Richness of their functional characteristics further revealed by their tolerance to salinity and wide range of pH. Organic farming system offers huge promise of achieving ecological, economic and social stability in food production system. However, organic farming is faced with a need to expand and develop in line with increasing demands for food and growing environmental concerns.

- The study concluded that three (03) STPGPB viz. *Enterobacter cloacae* (KP226569) (PR4), *Enterobacter* sp. (KP226570) (PR14) and *Azotobacter nigricans (*KP966496*)* (PR19) demonstrated their ability to enhance significantly seed germination and growth parameters of cereals in laboratory as well as field conditions.

- Additionally, selected three STPGPB significantly mitigated the negative impact of salt on growth parameters of cereal crops viz. rice, maize and millet in presence of ammonium sulfate (substitute of ACC/precursor of ethylene).

FUTURE PROSPECTS

- Potential PGPB may be further tested for improvement and enhancement of growth and yield of various other agricultural crops under both normal and abiotic stress conditions and development of technology for their applications at farmer's field.

- Potential PGPB may be further explored for production of efficient, cheap and convenient commercial product such as biofertilizer.

- Considering the crucial role of ACCD in mitigation of abiotic stresses, the enzyme may be subjected to detail investigations such as identification of gene(s) involved in its production and their biochemical, structural and functional characterization at molecular level.

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APPENDIX-A

1. Growth media

Different growth media were used in the present investigation is listed below. Composition of all the media in gL^{-1} is as follows:

1.1. Nutrient Agar Medium

Peptone	5.0
Beef extract	3.0
Sodium chloride	8.0
Agar	18.0
pH	7.0

1.2. YEM-Agar

This medium was used for the maintenance of Rhizobia isolates.

Yeast Extract	1.0 L
Mannitol	10.0
Dipotassium Phosphate	0.5
Magnesium sulphate	0.20
Sodium chloride	0.10
Agar	15.0
pH	7.0

1.3. YEM Broth Medium

This medium was used for the maintenance of Rhizobia isolates.

Yeast Extract	1.0
Mannitol	10.0

Dipotassium Phosphate	0.5
Magnesium sulphate	0.20
Sodium chloride	0.10
pH	7.0

1.4. King's B Medium

This medium is specific for fluorescent *Pseudomonas*.

Protease peptone	20.0
Glycerol	10.0 ml
K_2HPO_4 (anhydrous)	1.5
$MgSO_4.7H_2O$	1.5
Agar	18.0
pH	7.2

1.5. Pikovskaya Agar Medium

This medium was used for isolation of P-solubilizers.

Glucose	10.0
Tricalcium phosphate	5.0
$(NH_4)_2SO_4$	0.5
KCl	0.2
$MgSO_4.7H_2O$	0.1
$MnSO_4$	Trace
$FeSO_4$	Trace
Yeast extract	0.5
Agar	18.0
pH	7.0

1.6. Simmons Citrate Agar Medium

Sodium citrate	5.0
NaCl	5.0
$NH_4H_2PO_4$	1.0
K_2HPO_4	1.0
$MgSO_4.7H_20$	0.2
Agar	16.0
Bromothymol blue	0.08

1.7. Chrome Azurol S (CAS) Agar Medium

Modified Chrome Azurol S Medium was used for detection of siderophores.

Solution A

To prepare one litre of CAS medium, 60.5 mg Chrome Azurol S (CAS) was dissolved in 50 ml water and mixed with 10 ml Iron (III) solution (1mM $FeCl_3.6H_2O$, 10mM HCl). This solution was slowly added to 72.9 mg Hexadecyl - trimethyl ammonium bromide (HDTMA) dissolved in 40 ml water. The resultant dark liquid was autoclaved.

Solution B

Deferrated 1 M sucrose (30 ml), deferrated 100 ml of 10X basal salt medium containing K_2HPO_4 (1.0 g); NaCl (0.5 g); $NaMoO_4$ (0.005 g); $CaCl_2$ (1.0 g); PIPES (30.24 g) (1,4-piperazine di-ethane sulphonic acid) and Difco agar 15 g in 730 ml water was prepared. The pH of the medium was adjusted to 6.8 by the addition of NaOH (0.01*N*) before autoclaving. After cooling to 50 °C, 30 ml of deferrated casamino acid (10%) was added as sterile solution.

The solution A was finally added to solution B along the Glass wall with enough agitation to achieve maximum mixing without formation of foam. About 30 ml of medium was poured into each plate and stored in refrigerator.

Deferration

In studies involving siderophore, contaminating iron should be avoided to get good results, so treating the chemicals and Glassware for removal of iron, therefore becomes essential.

Removal of contaminating iron from Glass ware

Glass is a good ion exchange surface and hence may get contaminated with iron on the surface. All Glasswares used for siderophore study were soaked in 2*N* HCl for 24 hours and washed with double distilled water to remove the acid.

Removal of contaminating iron from media components

Media components such as sucrose, K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2$, NaCl and casamino acid were deferrated by extraction with 3% (w/w) 8-hydroxyquinoline in chloroform (Schwyn and Neilands, 1987).

1.8. Peptone Water

Peptone water was used for testing production of ammonia.

Peptone	10.0
NaCl	5.0
pH	7.0

1.9. Peptone Broth (g/l)

Peptone	0.1
NaCl	0.5
pH	6.0 ± 0.2

Autoclave at 15 lbs for 15 min

1.10. MacConkey Agar

Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000

Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
pH after sterilization(at 25°C)	7.1±0.2

1.11. Ashbys Mannitol Agar

Mannitol	20.000
Dipotassium phosphate	0.200
Magnesium sulphate	0.200
Sodium chloride	0.200
Potassium sulphate	0.100
Calcium carbonate	5.000
Agar	15.000
Final pH (at 25°C)	7.4±0.2

APPENDIX-B

2.0. Preparation of reagents and buffers

2.1 Citrate phosphate buffer

A. 0.2M Di sodium hydrogen phosphate	3.56 g
Dw	100 ml
B. 0.1 M Citric acid solution	1.92 g
Dw	100 ml

pH	A (ml)	B (ml)
5.0	51.5	48.5

2.2 DNS reagent

Sodium sulphate	0.05 g
Potassium sodium tartarate	27.0 g
3,5 Dinitrosalicylic acid	1 g
Sodium hydroxide	1 g
Phenol	0.2 ml
Dw	100 ml

Mix all above except sodium sulphate and store reagent in dark glass stopper bottle in refrigerator.

2.3 Sodium phosphate buffer

A. 0.1M sodium di hydrogen phosphate	1.2 g
Dw	100 ml
B. 0.1M Disodium hydrogen phosphate	1.78 g
Dw	100 ml

pH	A (ml)	B (ml)
5.7	93.5	6.5
6	87.7	12.3
7	39	61
8	53	94.7

2.4 CTAB buffer

Chemical	**Amount/ Concentration**	**Volume**	**Working Volume**
CTAB (Hexadecyl trimethyl-ammonium bromide)	10.0 g	100ml	20 ml
Tris-HCl pH 8.0	1 M	10.0 ml	10 ml
EDTA pH 8.0 (EthylenediaminetetraAcetic acid Di-sodium salt)	0.5 M	4.0 ml	4 ml
NaCl	5 M	28.0 ml	35 ml
H_2O	-	40.0 ml	29 ml
PVP 40 (polyvinyl pyrrolidone)	1 g		2ml

Adjust all to pH 8.0 and make up to 100 ml with H_2O.

2.5 TE buffer

Chemical	**Amount/** **Concentration**	**Volume**
Tris-HCl pH 8.0	10 mM	10 ml
0.5 M EDTA pH 8.0	1 mM	2 ml
H_2O		988 ml

2.6 50X TAE buffer

Chemical	**Amount/ Concentration**	**Volume**
Tris base	242 g	
Glacial acetic acid	0.5 M	57.1 ml
EDTA pH 8.0		100 ml
H_2O		850 ml

1X TAE buffer- 2 ml 50X TAE buffer and 98 ml distilled water.

2.7 1.5% Agarose gel

Agarose	1.5 g
1X TAE buffer	100 ml

APPENDIX-C

3.0. For biochemical test reagents for cereals crops

3.1. Protein estimation

2 % sodium carbonate in 0.1 N sodium hydroxide **(reagent A)**

0.5 % Cupper sulphate ($C_uSO_4.5H_2O$) in 1% potassium sodium tartrate **(reagent B)**

Alkaline cupper solution: mix 50 ml of A and 1 ml of B perior to use **(reagent C)**

Folin ciocalteau reagent **(FCR) (reagent D)-** reflux gently fo 10 hours a mixture consisting of 100 g sodium tungstate ($Na_2WoO_4.2H_2O$), 25 g sodium molybdate ($Na_2MoO_4.2H_2O$), 700 ml water, 50 ml of 85 % phosphoric acid and 100 ml of concentrated hydrochloric acid in a1.5 L flask. Add 150 g lithium sulfate, 50 ml water and few drops bromine water. Boil the mixture for 15 min without condenser to remove excess bromine. Cool, dilute to 1 L and filter. The reagent should have no greenish tint. (determine the acid concentration of reagent by titration with 1N NaoH to a Phenolphthalein end-point.)

3.2. Total carbohydrate content (g)

2.5 N HCl

Anthrone reagent : dissolve 200 mg anthrone in 100 ml of ice- cold 95% H_2So_4. Prepare fresh before use.

Standard glucose- dissolve 100 mg in 100 ml water. Working standard- 10 ml of stock diluted to 100 ml with distilled water. Store refrigerated after adding a few drop of toluene.

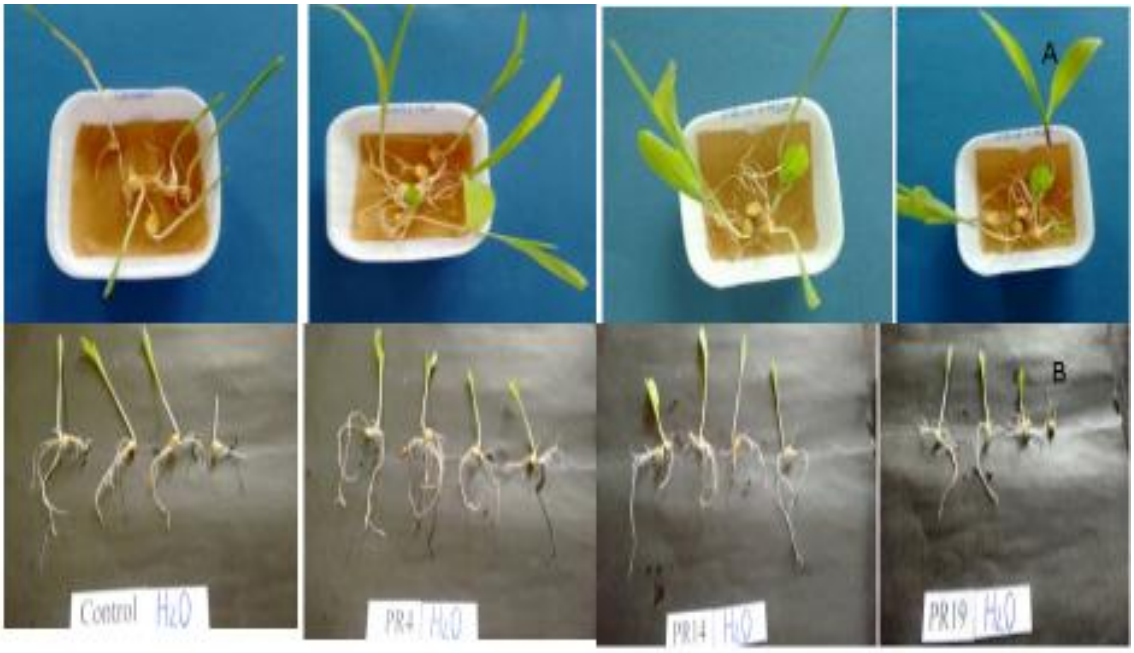

Photo 1. Effect of STPGPB on seed germination and growth parameters in maize varieties

A) SHIATS MS-2 B) Navjyot

Photo 2. Effect of STPGPB on seed germination and growth parameters of maize var SHIATS MS-2 under salt stress

Photo 3. Effect of STPGPB on seed germination and growth parameters of maize var Navjyot under salt stress

Photo 4. Effect of STPGPB on seed germination and growth parameters of maize var SHIATS MS-2 in presence of ammonium sulfate (substitute of ACC)

Photo 5. Effect of STPGPB on seed germination and growth parameters of maize var Navjyot in presence of ammonium sulfate (substitute of ACC)

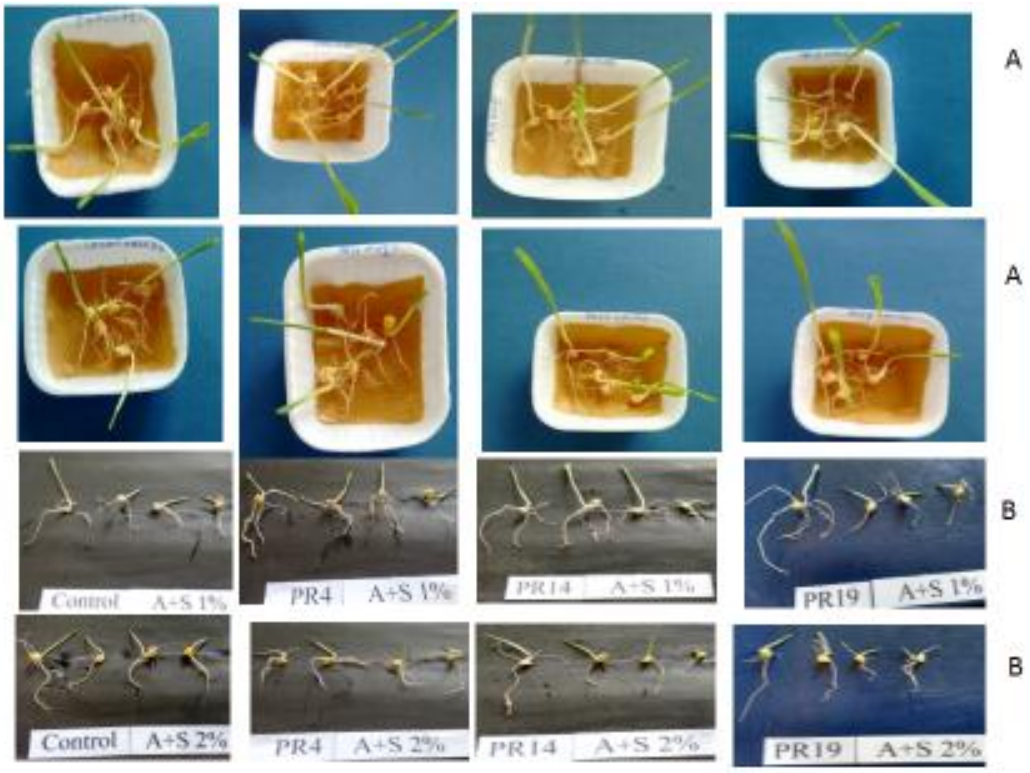

Photo 6. Effect of STPGPB on seed germination and growth parameters of maize in presence of ammonium sulfate (substitute of ACC) under salt stress A) SHIATS MS-2 B) Navjyot

Photo 7. Effect of *E. cloacae* (KP226569) on morphological and yield parameters of wheat var AAI-W6 under field condition

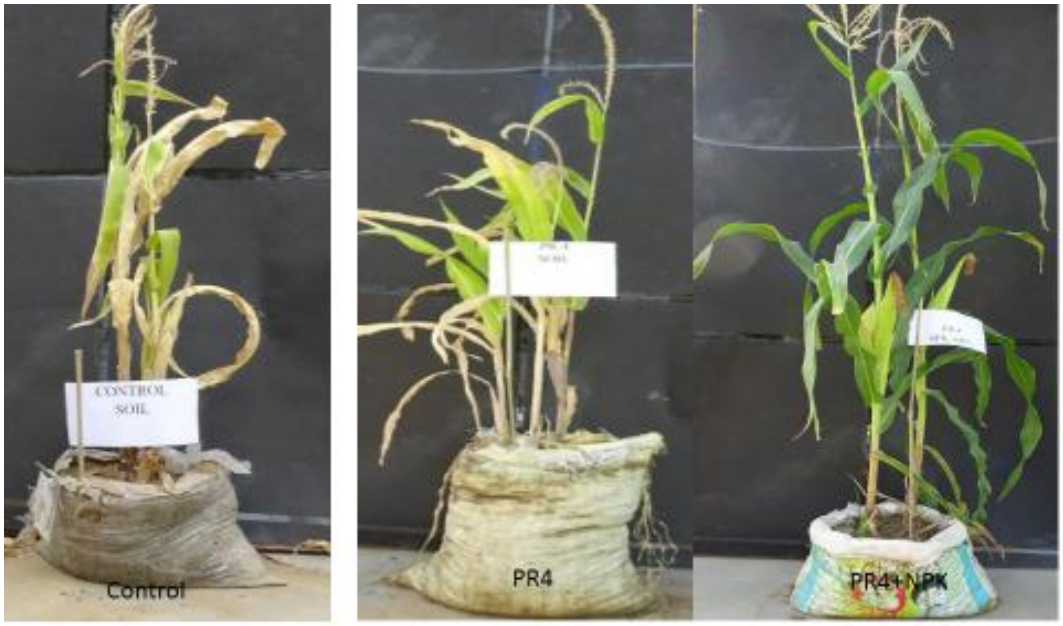

Photo 8. Effect of *E. cloacae* (KP226569) on morphological and yield parameters of maize var SHIATS MS-2 under field condition

Photo 9. SAC members : A) Prof. (Dr.)P. W. Ramteke (Advisor); B) Prof.(Dr.) M. P. Singh (External); C) Prof. (Dr.) John David (Chairmen); D) Prof.(Dr.) Rubina Lawrence (HOD and Member) E) Dr. P.K. Shukla (Co- Advisor); F) Dr. Rajesh Singh (Member)