Microbial consortia development and their evaluation for the growth of wheat under drought stress conditions

Thesis

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By

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Pantnagar
July 2018

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Authoress

C E R T I F I C A T E

This is to certify that the thesis entitled **“Microbial consortia development and their evaluation for the growth of wheat under drought stress conditions”** submitted in partial fulfilment of the requirements for the degree of **Master of Science** with major in **Microbiology** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bona-fide research carried out by **Ms. Manisha Panwar, Id. No. 51204,** under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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C E R T I F I C A T E

We, the undersigned, members of the Advisory Committee of **Ms. Manisha Panwar, Id. No. 51204,** a candidate for the degree of **Master of Science** with major in **Microbiology** agree that the thesis entitled **"Microbial consortia development and their evaluation for the growth of wheat under drought stress conditions"** may be submitted in partial fulfilment of the requirements for the degree.

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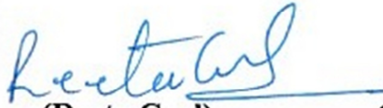

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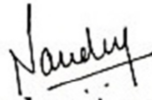

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Introduction

INTRODUCTION

In natural environmental conditions biotic and abiotic stresses readily affect the growth and overall productivity of the crop plants. The abiotic stresses are about to increase in the near future because of global climate change. Conditions of abiotic stresses might be the major limiting factors observed for crop cultivation around the world. This mainly concerns about drought, salinity and high temperature, prominent causes of drop off worldwide crop productivity. In arid and semi-arid areas, drought rigorously affects the overall growth and productivity of crop plants and expected to spoil 50% of the arable lands by 2050 **(Vinocur and Altman, 2005).** Growth and productivity of plant collapses mainly under drought stress conditions **(Lafitte *et al.*, 2007)**. The reduced plant growth were observed in various staple food crops such as wheat **(Rampino *et al.*, 2006),** rice **(Lafitte *et al.*, 2007),** and maize **(Kamara *et al.*, 2003).** Drought influences the overall plant growth and development by affecting water potential, obstructing common metabolism of cells and by changing its activity at cellular, sub-cellular and entire plant levels which consequences in specific and nonspecific physiological responses. Due to this abiotic stress, plants also experience nutrient imbalances as a result of deprived nutrient accessibility and transport from soil (source) to root hairs (sink). Drought stress conditions also provoke the formation of free radicals in form of reactive oxygen species (ROS) including hydrogen-peroxide, superoxide radicals and hydroxyl radicals stress. High amount of ROS can cause damage at various levels, like lipid peroxidation, membrane deterioration, degradation of proteins, lipids and nucleic acids in plants **(Nair *et al.*, 2008).** Thus drought as a multidimensional stress which negatively affects growth and productivity of plants and instigates the problem of food security.

In India about two-third population depends upon agriculture, hence drought conditions are likely to threaten the overall economy of the country. Most of the agricultural land in India being rainfed and it is heavily dependent on climate and monsoon rainfall for yield. Furthermore, increased greenhouse gas emissions are expected to cause general drying of the subtropics by the end of this century and causing widespread drought **(IPCC, 2007).** The negative effect of abiotic stress on

plant growth and development are evident among the emerging ecological impacts of climate change **(Bellard *et al*., 2012),** and the constraints to crop production worsened with the increasing human population competing for environmental resources **(Wallace *et al*., 2003).**

Wheat is one of the major staple food worldwide, but almost 32% of wheat cultivars face up to various types of drought stresses during the growth season **(Morris *et al*., 1991).** Presently, wheat is the largest staple food in Asia, this region has a broad arid and semiarid area and drought is the major factor for losses of wheat yield. Wheat is known as second most important food crop in the developing world after rice. Water stress conditions during grain development causes large yield losses in wheat. This reduction is mainly accounted by a reduction in starch accumulation, since in general over 65% of cereal dry weight is accounted by starch **(Duffus, 1992).** In recent years, the level of wheat production could not fulfill the satisfactory demand, prompting price instability and hunger riots. The predicted world population by 2050 would be around 9 billion, which increases the demand for wheat and expected to increase by 60%. Wheat provides essential nourishment for more than 1/3 world population and crop yield will be considerably influenced in the perspective of global climate change and drought stress **(Chaves and Oliveira, 2004).** Therefore, it is quite important to alleviate the adverse effects of drought stress on wheat growth **(Krugman *et al*., 2011; Zhong and Shangguan, 2014).** Various strategies such as traditional breeding and genetic engineering of drought-tolerant transgenic plants have been incorporated to enhance the capability of plants to bear drought stress conditions, but implementation of these methods are difficult to apply as they are extremely technical and labor-intensive. Therefore, application of microorganisms in drought pretentious agricultural lands could be a profound and alternative approach.

Plant growth promoting rhizobacteria (PGPR) could play an important and significant role in mitigation of drought stress in plants. PGPR have received great importance for improvement in plant tolerance against salinity, drought, cold and heavy metal toxicity **(Pinedo *et al.,* 2015; Singh and Singh, 2017).** Root colonizing plant growth promoting rhizobacteria (PGPR) exhibited reflective enhancement in plant growth and decreased susceptibility to diseases caused by several plant pathogenic organisms such as fungi, bacteria, viruses and nematodes **(Kloepper *et al*., 2004).**

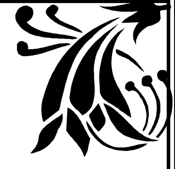
Moreover, root and plant tissues associated microorganism exert multiple beneficial traits, and can participate into the alleviation of plant stress through various mechanisms. Plant growth promoting bacteria (PGPB) can contribute to alleviate abiotic stresses of host plants via a variety of mechanisms such as decrease in ethylene level by 1-aminocyclopropane-1 - carboxylate (ACC) deaminase production. The production of exopolysaccharides (EPS) can also play an important role in supporting plant growth under water deficit and salt stress conditions. Other metabolites produced by PGPB are potentially involved in supporting plant growth under stress conditions include siderophores, phytohormones, organic acids and volatile compounds. Additionally, these bacteria may enhance plant stress tolerance by inducing accumulation of osmolytes, antioxidants, regulation of stress responsive genes, increase in photosynthesis and alteration in root morphology **(Tiwari *et al.,* 2016; Vurukonda *et al.,* 2016; Singh and Singh, 2017; Singh *et al.,* 2017).**

Recently, besides using single strain of plant growth promoting rhizobacterium, co-inoculation of two or more PGPR strains has been experimented to achieve prominent multifarious effect in sustainable agriculture system. Beneficial microorganisms with different attributes can be used as consortium, which can work synergistically promoting each other's beneficial effects along with plant health. The ability of microorganisms to confer habitat-specific stress tolerance to host plants elicits an idea to develop consortia of beneficial microorganisms possessing plant growth enhancing traits with drought tolerance ability. Formulation and development of biofertilizers by using PGPR and their consortia is an efficient mean for applying microorganisms in field conditions and their effective performance for plant growth. The performance of PGPR strains in field conditions greatly depends on proper means of delivery, application strategy and persistence of the strains in plant system and rhizospheric soil. It is generally noted that the performance efficacy of the rhizosphere strains decreases in field conditions as compare to *in vitro* conditions. To overcome this issue biofertilizers can be applied in soil, seeds and in foliar sprays, hence efficient delivery plays a vital role in the performance of the rhizobacterial strains in field conditions **(Vidhyasekaran and Muthamilan, 1999).** Inert carrier based bioformulations are helpful as delivery system for both foliar and soil application and enables easy-handling, long-term storage and high effectiveness. Bioformulation developed with a suitable carrier also enhances the shelf

life of microbial products such as antibiotics, siderophores, hydrolytic enzymes, phytohormones and volatile extracellular metabolites **(Ardakani *et al*., 2010; Chen *et al*., 2000; Jorjani *et al.*, 2011; Shahraki *et al*., 2009).** Therefore, development of a stable carrier based bioformulation is important to serve areas where there is subsistent farming and plant diseases are major problems **(Ardakani *et al*., 2010).** Therefore, there is an immense need of characterizing drought tolerant PGPRs and developing their consortium based approach in order to apply them in form of biofertilizers for gaining improvement in total plants growth and yield, and as well to confer drought tolerance in plant under drought conditions.

Considering the importance of PGP microbial consortium under drought stress, the present study is designed with following specific objectives:

- Retrieval and authentication of bacterial cultures from departmental culture collection.
- Screening of retrieved bacterial isolates on the basis of polyethylene glycol (PEG) tolerance level and their characterization.
- Development of bacterial consortia based on the compatibility of selected potential strains.
- Exploration of *in situ* plant growth promoting potential of selected consortia on wheat under drought stress conditions.



Review of Literature

Drought is one of the major environmental stresses that limit crop growth and productivity worldwide, while global warming and water scarcity will further make the situation worse. As an extreme event (abiotic factor), drought severely affects food production. Considering climate change and anthropogenic influences, an overall enhanced drought risk for crop yield in the future is well documented. In India, this risk is greater due to deviated monsoon rains, depleted groundwater, and the pressure of food demand from a population of 1.252 billion. More regional droughts in the agriculturally important southern coast India, central Maharashtra and Indo-Gangetic plains were highlighted indicating higher food security and socioeconomic vulnerability **(Wu *et al.,* 2004; Sheffield *et al.,* 2014; Lesk *et al.,* 2016). Wilhite and Glantz (1985)** categorized drought into: *meteorological drought*, *hydrological drought*, *agricultural drought* and *socioeconomic drought*. The first three approaches deal with ways to measure drought as a physical phenomenon. Socioeconomic drought occurs when the supply of an economic goods and services such as food, drinking water and energy are reduced as a result scarcity in water supply due to changes in meteorological and hydrological drought conditions.

During the conditions of drought, plant's metabolic processes and transpiration exceeds the water availability for absorption or when water content of plant gets low enough to interfere with normal plant processes, water deficit/stress is created. It can also result from reduced moisture of soil, due to less rainfall or supplemental irrigation. Water stress has been found as an important factor affecting deleteriously various stages/metabolic processes of plants **(Upadhyay and Panda, 2013).** For example, water stress reduces the water potential of plant cell and thus enhances the solute concentration, which further hinders cell enlargement, stem proliferation, and root elongation, thereby hampering the plant growth **(Akinci and Losel, 2012).** However, when plants are growing under stressed situation, it exhibits visible symptoms. Wilting is the condition of plants where the non-wooden parts of the plants become non rigid due to low turgor pressure and is one of the most common symptoms of water stress **(Correia *et al.*, 2001; Cabuslay *et al.*, 2002).** Water stress may also cause stomata

closure. Accumulation of plant hormone, for instance, abscisic acid (ABA), is responsible for the stomatal closure **(Socias *et al.*, 1997).** This further reduces gaseous exchange, decrease in photosynthesis and pigmentation ROS accumulation. Stomata closure under drought stress is also found to be related to alter nutritional status, xylem sap pH, and hydraulic conductivity as well as declines water content in leaf **(Oren *et al.*, 1999).** Summarily, drought stress interrupts the enzymatic reactions mainly involved in CO_2 fixation and ATP synthesis and thus affects the plant by altering (1) photosynthesis, (2) transpiration, (3) nutrient uptake, (4) hormone production, (5) homeostasis and (6) other metabolic processes. Moreover, a decline in relative water content (RWC) reflects a loss of turgor that results in limited cell expansion and consequentially, reduced plant growth **(Ashraf, 2010; Lu *et al.,* 2010). Jarvis and Jarvis (1963)** reported that species that are better adapted to dry environments have high RWC. Proline is one of the most important osmolytes that accumulate in plants experiencing drought stress **(Yoshiba *et al.,* 1997; Huang *et al.,* 2014).** Proline contributes to stabilizing sub cellular structures (e.g. proteins and membranes), scavenging free radicals and buffering cellular redox potential **(Hayat *et al.,* 2012).**

2.1 Strategies to combat drought stress

Drought stress conditions can alter metabolism, growth and development of plants. Worldwide extensive research is being carried out to develop strategies to cope with drought stress through development of drought tolerant varieties by conventional breeding and biotechnological approaches. Most of these technologies are cost-intensive, require much more time and labour. The other sustainable method of inoculation of plant growth promoting rhizobacteria can also help plants to cope with drought stress and enhancing plant growth promotion, nutrients management, crop productivity, disease control and providing stress resistance **(Grover *et al.*, 2010).**

2.2 Plant growth promoting rhizobacteria

Plant-growth-promoting rhizobacteria (PGPR) are a group of microbes which colonize the plant roots and improve plant growth either directly or indirectly. Various studies have been already published that explain the effect of PGPR in relieving abiotic stress in different crop plants. There are diverse PGPR-induced changes in plants, and growth promotion perhaps results due to a complex combination of various PGPR-

induced mechanisms that affect both plant development as well as plant nutrition. PGPR interact with the roots of plants and consequently stimulus plant health and soil fertility. PGPR inhabit around the root surface, involved in growth promotion and development of plant directly or indirectly by secretion of various regulatory chemicals in the vicinity of rhizosphere **(Ahmad *et al.,* 2008)**. Rhizospheric microorganisms can improve plant performance under stress environment and result in the enhance crop yield. Plants in their natural environment are colonized by intracellular and endocellular microorganisms **(Gray and Smith, 2005).** Some PGPR provides plants with fixed nitrogen, phytohormones and iron that have been sequestered by bacterial siderophores and soluble phosphate, thisdirectly enhance plant growth and development **(Rodriguezand Fraga, 1999; Hayat *et al*., 2010).** Others PGPR do this indirectly by protecting the plant against soil-borne diseases, most of which are caused by pathogenic fungi **(Lugtenberg and Kamolova, 2009).** In addition to possessing general plant growth promoting properties such as production of indole-3-acetic acid (IAA), siderophores, 1 -amino-cyclopropane-1 -carboxylate (ACC) deaminase, hydrogen cyanate (HCN), nitrogenase and phosphate solubilization **(Fig 2.1)**, some PGPR also possess more environment specific plant growth promoting (PGP) traits such as heavy metal detoxifying activity, salinity tolerance, and biological control of phytopathogens and insects **(Singh *et al.,* 2010; Liu *et al.,* 2016; Singh and Singh, 2017).**

2.3 Plant microbe interaction in rhizosphere

Plants and microbes can have interactions including pathogenic, symbiotic and associative. These interactions affect plant growth, productivity, stress tolerance and disease resistance. Interactions between plants and microbes may occur at phyllosphere, endosphere and rhizosphere. Production of more root exudates by plant favors maximum microbial population in rhizosphere, which is an ecologically significant region. For the better acquisition of water and nutrients by plants and for beneficial interactions of plants with soil-borne microorganisms the environment around rhizosphere is an essential factor **(Ryan *et al*., 2009).** Biogeochemical cycling of nutrients like C, P, N and S, removal of toxins, production of several phytohormones and antibiotics all are associated with rhizospheric microbial communities **(Cardoso and Freitas, 1992)**. Microbes can interact with each other for nutrient sources as one microbe may convert plant exudates into a form that can be used by another microbe.

Thus, rhizosphere is a dynamic and versatile ecological environment of strong plant-microbe interactions **(Mayak *et al.*, 2004).**

2.4 Mechanisms of PGPR

PGPR regulate plant growth promotion directly by the production of phytohormones like auxins, cytokinins, gibberellins and by solubilization of minerals like phosphorus and zinc, production of siderophores and enzymes they support in enhancement of plant nutrition (**Bhattacharyya and Jha, 2012**). Indirect mechanism involves the ability of PGPR to reduce the deleterious effects of plant pathogens that inhibit plant growth, including antibiotic production, parasitism, competition for nutrients and niches within the rhizosphere, synthesis of extracellular enzymes including chitinase, cellulose, proteases and lipases to hydrolyze the fungal cell wall, decreasing pollutant toxicity (**Zahir *et al.*, 2003; Podile and Kishore, 2006; Fig 2.1).**

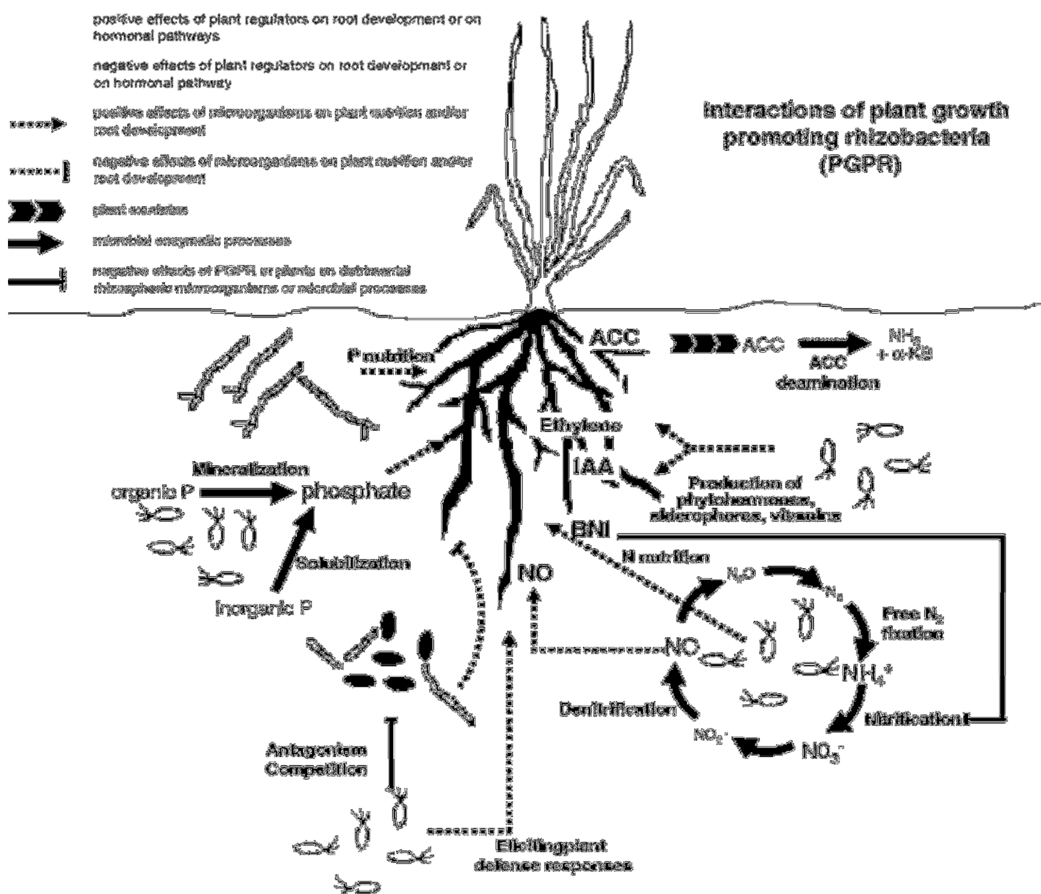


Fig 2.1: Plant growth promotion mechanisms (positive and negative effects) associated with soil and rhizoshere microorganisms (Source: **Richardson *et al.*, 2009**)

2.4.1 Phosphate solubilization

Phosphorus, second most important plant growth-limiting nutrient, which abundantly available in soils in both inorganic and organic forms **(Khan *et al.*, 2009).** Phosphorus is one of the most essential plant nutrients which profoundly affect the overall growth of plants **(Wang *et al.*, 2009)**. Despite of large reservoir of P, it is generally available in low amount in plants because majority of P present in the soil is found in insoluble forms, while the plants are able to absorb it only in two soluble forms, the monobasic (H_2PO^{-4}) and the diabasic $(HPO_2)^{-4}$ ions. To overcome the phosphorus deficiency in soils applications of phosphatic fertilizers in agricultural fields is necessary. Very low amount of phosphatic fertilizers applied in the field is absorb by plants and the remaining is rapidly converted into insoluble complexes in the soils **(Mckenzie and Roberts, 1990).** Regular application of phosphate fertilizers is costly and environmentally undesirable. For improving plant growth and crop yield in low P soils an ecologically safe and economically reasonable approach has to be developed. In this context, microorganisms such as PGPR possessing phosphate solubilizing activity, often termed as phosphate solubilizing microorganisms **(Fig 2.1)**, may provide the available forms of P to the plants and hence a viable substitute to chemical phosphatic fertilizers **(Richardson *et al.*, 2009).** According to **Zaidi *et al.* (2009)** phosphate solubilizing bacteria (PSB) are considered as most promising biofertilizer in compare with other mechanisms to mineralize phosphorus. The mineralization of organic forms of phosphorus occurs by the production of variety of different enzymes such as phosphatases, catalyzing the hydrolysis of phosphoric esters **(Glick, 2012).** Typically, the solubilization of inorganic phosphorus occurs as a consequence of the action of low molecular weight organic acids which are synthesized by various soil bacteria **(Zaidi *et al.*, 2009).**

2.4.2 Zinc solubilization

Zn is another one of the essential micronutrients required for optimum plant growth and plays a vital role in cell metabolism and required in relatively low concentrations in plant tissues (5–100 mg Kg^{-1}). It is also the co-factor and metal activator of many enzymes **(Desai *et al.*, 2012).** Plants can uptake zinc as divalent cation **(Kabata-Pendias and Pendias, 2001)** but only a very minor portion of total zinc is present in soil solution as soluble form and remaining zinc present in the form

of insoluble complexes and minerals **(Alloway, 2008).** Due to low solubility of Zn in soil Zn-deficiency is well reported in the soils of around the world. Zn in soils associated mainly with hydrous Fe^{3+} and Al^{3+} oxides and with clay minerals. So, for increasing availability of Zn in soil, release of insoluble and fixed forms of Zn is important, which can be done with the help of microorganisms that are able to solubilize insoluble Zn salts through the production of organic acids **(Venkata Krishnan *et al.*, 2003; Bhupinder *et al.*, 2005). Quareshi *et al.* (2010)** reported that all plant growth parameters of legume crop increase when zinc solubilizing bacteria were co-inoculated in the seedlings as compared to uninoculated controls. Various workers shown that PGPR can enhance growth and zinc content when inoculated in plants. These Zn solubilizing PGPR include *Pseudomonas*, *Rhizobium* **(Deepak *et al.*, 2013; Naz *et al.*, 2016),** *Bacillus aryabhattai* **(Ramesh *et al.*, 2014),** *Bacillus sp.* **(Hussain *et al.*, 2015)** and *Azospirillum.* **Vaid *et al.* (2014)** reported rice growth promotion with 42.7% increased zinc content in grains when inoculated with zinc solubilizing bacteria.

2.4.3 Siderophore production

Iron (Fe) is an essential plant micronutrient and served as a cofactor of many enzymes present in plants. At physiological pH (7.35–7.40), the ferrous form (Fe^{2+}) of iron is soluble, while the ferric form (Fe^{3+}) is insoluble **(Bou Abdallah, 2010)** and form insoluble hydroxides and oxyhydroxides which is not available to both plants and microorganisms **(Rajkumar *et al.*, 2010).** In order to survive under such Fe-depleted environment, microorganisms produce siderophores, organic compounds with low molecular masses **(Ahmed and Holmstrom, 2014).** Siderophores are the metal-chelating agents that primarily function to capture the insoluble ferric iron from different habitats **(Nagoba and Vedpathak, 2011).** Microbial siderophores enhance iron uptake by plants, as they recognize the bacterial ferric-siderophore complex and enhance iron uptake by plants and thus plays an important role in plant growth promotion **(Fig 2.1)**. Siderophore is produced by several PGPR such as *Bacillus* **(Kloepper *et al.*, 1980; Katiyar and Goel, 2004; Singh *et al.*, 2008; Dimkpa *et al.*, 2009),** *R. leguminosarum* bv. *viciae* **(Rogers *et al.*, 2000)** etc**. Gangwar and Kaur (2009)** reported that *Escherichia coli* from endo-rhizosphere of sugarcane (*Saccharum* spp.*)* and rye grass (*Lolium perenne*) is associated with maximum siderophore

production and thus enhances plant growth considerably. **Chakraborthy *et al.* (2006)** isolated *Bacillus megaterium* from rhizosphere of tea and analyzed its abilities to improve plant growth and disease reduction in tea plants. *Trichoderma asperellum* was found to produce siderophore which had a potential role in enhancing cucumber growth by ameliorating salt stress **(Qi and Zhao, 2013).**

2.4.4 Nitrogen fixation

Nitrogen (N) is another most essential nutrient for plant growth and productivity. It is required for cellular synthesis of enzymes, DNA, RNA, proteins and chlorophyll. 78% of N is present in atmosphere in free state, but is unavailable to the plants which needs to be converted into ammonia that can readily absorb by the plants. There are some specialized group of microorganism that can convert the atmospheric nitrogen into plant-utilizable forms such as ammonia known as nitrogen-fixers or diazotrophs by the process of Biological N_2 fixation (BNF) **(Kim and Rees, 1994).** Nitrogen fixing organisms are generally categorized as (a) symbiotic N_2 fixing bacteria which forms symbiosis with leguminous plants (e.g. rhizobia) **(Zahran, 2001; Ahemad and Khan, 2012)** and non-leguminous trees (e.g. *Frankia*) and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing forms such as cyanobacteria (*Anabaena, Nostoc*), *Azospirillum, Azotobacter, Gluconoacetobacter diazotrophicus* and *Azocarus* etc **(Antoun *et al*., 1998; Riggs *et al*., 2001).** BNF represents an economically beneficial and environmentally sound alternative to chemical fertilizers **(Ladha *et al*., 1998).** The nitrogenase protein complex carries out the process of N_2 fixation **(Kim and Rees, 1994)** is a metallo-enzyme consisting of (i) dinitrogenase reductase which is an iron protein and (ii) dinitrogenase consists of a metal cofactor. Dinitrogenase reductase provides electrons with high reducing power while dinitrogenase uses these electrons to reduce N_2 to NH_3 **(Halbleib and Ludden, 2000). Chowdhury *et al.* (2007)** found high nitrogenase activity by acetylene reduction assay from the bacterial isolates which were isolated from the roots of *Lasoiurus sindicus*. **Minamisawa *et al.* (2004)** found that the endophytic *Clostridium* spp. which was isolated from the graminaceous plants showed the nitrogenase activity in the range of 16-24 nM ethylene produced h^{-1}.

2.4.5 Phytohormones production

Phytohormones production by PGPR is considered to be an important mechanism by which the bacteria promote plant growth and productivity **(Vessey, 2003).** With the

production of different phytohormones like indole-3-acetic acid (IAA), gibberellic acid and cytokinins, PGPR help in the growth and development of plants as these phytohormones promote increase of root surface and length **(Han *et al.*, 2005; Fig 2.1)**. Indole-3-acetic acid (IAA) is one of the most common and naturally occurring auxin which is known to be involved in root initiation, cell division, cell enlargement, differentiation of phloem and xylem, and delaying of leaf senescence **(Glick, 1995). Patten and Glick (1996)** reported that about 80% rhizospheric microorganisms are able to produce auxins as secondary metabolites**.** IAA is synthesized through L-tryptophan metabolism by plants and many PGPR, algae and fungus. **Karnwal (2009)** studied that the production of IAA increased with the increase in concentration of tryptophan in two strains of fluorescent *Pseudomonas.* **Patten and Glick (2002)** and **Gravel *et al.* (2007)** demonstrated that the rhizospheric microorganism such as *Pseudomonas* spp. and *Rhizobium* sp. are associated with their potential to enhance plant growth by the production of IAA. IAA production by bacteria depends upon the factors like carbon and nitrogen source present in the medium, with supplement of tryptophan, pH and temperature **(Mohite, 2013).** Gibberellins (GAs) are plant hormones that regulate plant growth and influence stem elongation, germination, dormancy, control flowering, leaf and fruit senescence, enzyme induction and sex expression. Cytokinins have important role in resistance to plant pathogens **(DelloIoio *et al.*, 2007)**. **Arkhipova *et al.* (2007)** experimentally determined that the inoculation of plant with cytokinin producing bacteria stimulate shoot growth and reduce the root to shoot ratio. Several workers reported the production of gibberellins and cytokinin by some species of PGPR **(Tien *et al.*, 1979; Akiyoshi *et al.*, 1987; Cacciari *et al.*, 1989)**. Production of indole-3-acetic acid (IAA) by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* **(Bastian *et al.*, 1998),** zeatin and ethylene by *Azospirillum* sp. **(Strzelczyk *et al.*, 1994)**. PGPR can influence the defense system of plants by synthesizing salycilic acid **(De Vleesschauwer *et al.,* 2008; Visca *et al.,* 1993).** This allows plants to activate some genes in their defense system that are involved in systemic acquired resistance. Thereby, PGPR reveal their universality. Thus, synthesizing salycilic acid by *Pusedomonas fluorescens* CHAO **(Maurhofer *et al.,* 1994)** and *Pusedomonas aeruoginosa* 7NSK2 **(De Meyer *et al.,* 1999)** strains could launch SAR reaction in tobacco, but they did not show such effect in mutant NahG plants characterized by the ability to rapidly degrade it. It is interesting that *P. aeruoginosa* strain 7NSK retained such ability on rice plants, while protecting them from *Magnaporthe oryzae* **(De Vleesschauwer *et al.*, 2008).**

2.4.6 Production of antibiotics

Many PGPR have the ability to produce peptide antibiotics, considered one of the most powerful biocontrol tools that inhibit synthesis of pathogens cell walls, influence membrane structures of cells, inhibit the formation of initiation complex on small subunit of ribosomes **(Maksimov *et al.*, 2011)**. A large number of antibiotics have been identified produced by different microorganisms like pseudomonads able to produce amphisin, 2, 4- diacetyl flouro glucinol, hydrogen cyanide, oomycin A, phenazine, pyoluteorin, pyrrolnitrin, tensin, tropolone and cyclic lipopeptides, however *Bacillus, Streptomyces,* and *Stenotrophomonas spp. produce* oligomycin A, kanosamine, zwittermicin A, and xanthobaccin **(Compant *et al.*, 2005).** Antibiotics produced by *Bacillus* sp. are active with both Gram positive and Gram negative bacteria and also active against pathogenic fungi *Alternaria solani, Aspergillus flavus, Botryos phaeriaribis, Colletotrichumg loeosporioides, Fusarium oxysporum, Helminthosporium maydis, Phomopsis gossypii, etc.* (**Maksimov *et al.*, 2011**). It was reported that *B. subtilis* surfacine stimulate induced systemic resistance through activation of components like lipoxygenases, lipid peroxidases and the formation of reactive oxygen species (**Maksimov *et al.*, 2011, Ongena *et al.*, 2007**). **Hill *et al.* (1994)** reported that *P. fluorescens* BL915 strain produce Pyrrolnitrin antibiotic which was able to prevent the damage of *Rhizoctonia solani* during damping-off of cotton.

2.4.7 Lytic enzyme production

Microorganism produce lytic enzymes that can hydrolyze polymeric compounds like cellulose, hemicellulose, chitin and protein and directly suppress the growth and activities of pathogens. Hydrolytic enzymes such as chitinases, proteases, lipases and glucanases are involved in fungal cell wall lysis. Hydrolytic enzymes directly contribute in the parasitization of phytopathogens and rescue plant from biotic stresses. Some bacterial strain of *Bacillus* and *Pseudomonas* sp. secretes chitinases and glucanases and able to suppress growth and development of fungi. Use of chitinases producing bacteria for biological protection of crops from pathogens, especially those that contain chitin and glucans within their cell wall structure, is the most prominent approach in the agriculture **(Maksimov *et al.*, 2011).** Hydrolytic enzymes prevents plant diseases **(Van Loon, 2007)** by causing lysis of deleterious

microbes in the close vicinity of the plant as they secretes increased level of cell wall lytic enzymes (chitinases, glucanases and proteases) **(Raval and Desai, 2012).** Production of lytic enzymes by rhizospheric bacteria involved in the control mechanisms against plant root pathogens including *Fusarium oxysporium* and *Rhizoctonia solani* **(Chaiharn *et al*., 2008).** Cell walls of most plant pathogenic fungi (except Oomycetes) are made up of chitin, which is a homo polymer of N-acetyl glucosamine residues linked in β-1, 4 linkage. Chitinases which hydrolyse this polymer are produced by various organisms and have been implicated in the control of fungal diseases. Inactivation of genes involved in their biosynthesis has been used to provide evidence for their contribution in biocontrol in plant **(Kobayashi *et al*., 2002).** The soil borne fluorescent *Pseudomonas* has capacity to produce a wide range of enzymes and metabolites like chitinase, protease/elastase and β-1, 3glucanase which contribute for its antagonistic or biocontrol activity **(Kavino *et al*., 2008)**. These enzymes are supposed to degrade the cell wall of various bacterial and fungal plant pathogens. *Myxobacterium produces lytic enzymes* which are effective in the suppression of fungal plant pathogens **(Kobayashi and El-Barrad 1996; Bull *et al*., 2002).** *Serratia marcescens* reported to reduce mycelial network of *Sclerotium rolfsii* by expressing chitinase **(Ordentlich *et al*., 1988).**

2.4.8 Exopolysaccharide (EPS) production

EPS are most important part of extracellular matrix that often represent 40–95% of bacterial weight **(Flemming and Wingender, 2001).** Bacteria produce EPS in two forms slime EPS and capsular EPS **(Vanhooren and Vandamme, 1998).** EPS play an important role in microbial aggregation, plant–microbe interaction, and bioremediation **(Manca de Nadra *et al*., 1985).** Bacterial EPS are hydrated compounds with 97% of water in polymer matrix which impart protection against desiccation **(Wingender *et al*., 1999; Bhaskar and Bhosle, 2005; Hunter and Beveridge, 2005)**. EPS produce by *Pseudomonas* help it to survive under drought stress conditions **(Sandhya *et al*., 2009).** The EPS protect these bacteria from desiccation under drought stress by enhancing the water retention and by regulation of organic carbon source's diffusion **(Roberson and Firestone, 1992; Chenu, 1993; Chenu and Roberson, 1996).** EPS producing *Azospirillum* treated plants showed resistance to water stress **(Bensalim *et al*., 1998)** by the improvement in the soil structure **(Sandhya *et al*., 2009)**. **Alami *et al*. (2000)**

showed that under drought stress sunflower plants inoculated with EPS-producing rhizobial strain YAS34 strain showed increase in root tissue.

2.4.9 ACC deaminase

Ethylene is required for normal growth of plants. It is involve in seedling emergence, leaf and flower senescence, fruit ripening and lateral bud differentiation. Overproduction of ethylene hormone has inhibitory effects on root development and may lead to abnormal growth of the plants. PGPRs having ACC deaminase activity help plants to withstand several abiotic and biotic stresses by reducing the level of ethylene **(Dimkpa *et al*., 2009; Zahir *et al*., 2009; Mayak *et al*., 2014).** Bacteria such as *Alcaligenes* sp., *Bacillus pumilus, Pseudomonas* sp., *Azospirillum* sp., *Gluconacetobacter diazotrophicus, Variovora xparadoxus, Azorhizobium caulinodans, Herbaspirillum* spp. and *Burkholderia vietnamiensis* are able to produce ACC deaminase enzyme **(Dobbelaere *et al*., 2003).** PGPR possess the enzyme ACC deaminase can cleave ACC, the immediate precursor of ethylene in plants, to α-ketobutyrate and ammonia **(Honma and Shimomura, 1978).** The products of this hydrolysis are used by the ACC-degrading bacteria as nitrogen and carbon sources resulting lower ethylene level in stressed plant **(Fig 2.2)**. **Mayak *et al*. (2004)** isolated the ACC deaminase-containing bacterium *Achromobacter piechaudii* from a rhizosphere of *Lyciums hawii* plant and inoculated it with three-week-old tomato plant seedlings, no watering was for one week and then re-watered every other day for a week and found that the inoculated treatment had four times the biomass and one quarter the level of ethylene of plants as compare to the uninoculated treatment. **Arshad *et al*. (2008)** reported that drought stress in pea plants is reduced when treated with *Pseudomonas* spp. which has the ability to produce ACC deaminase. Several workers demonstrated the efficacy of protecting a range of different plants against loss of biomass from drought stress using ACC deaminase-containing plant growth-promoting bacteria **(Zahir *et al*., 2008; Belimov *et al*., 2009; Shakir *et al*., 2012).** All of the above study concluded that higher level of ACC deaminase production by microorganism improves plant growth and drought tolerance.

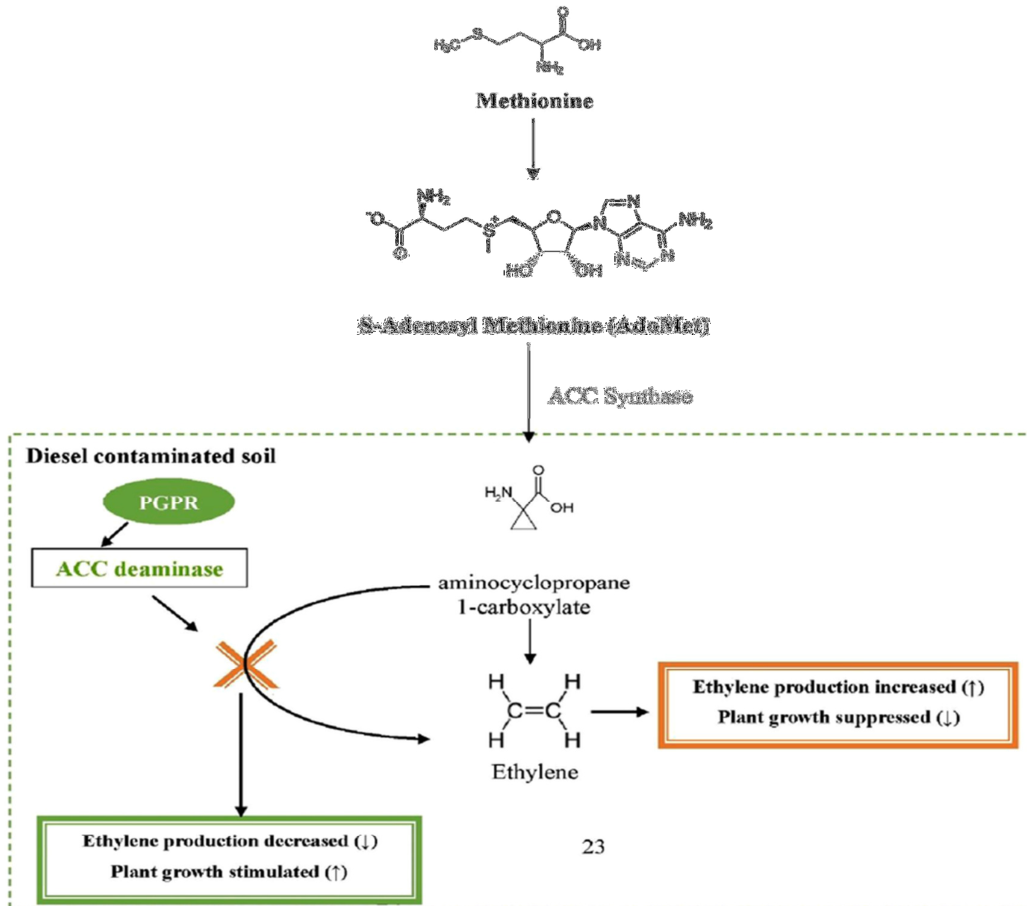


Fig 2.2: Schematic representation of ACC deaminase activity to prevent ethylene based inhibition of root elongation (Source: **Arslan *et al.*, 2014**)

2.4.10 Osmotic adjustment for drought stress

Plants adaptation to drought stress is associated with metabolic adjustments that lead to the accumulation of several osmolytes like proline, sugars, polyamines, betaines, quaternary ammonium compounds, polyhydric alcohols and other amino acids and water stress proteins like dehydrins **(Yancey *et al.*, 1982; Close, 1996).** Solutes are important in maintaining plant cellular turgor and adaptation to harsh environments **(Drabble and Drabble, 1907)**. **Kemble and Macpherson, (1954)** demonstrated that several amino acids, most prominently proline, were synthesized *de novo* upon wilting. In response to drought stress PGPR secrete osmolytes, which act synergistically with plant-produced osmolytes and enhanced plant growth **(Paul *et al.*, 2008). Sandhya *et al.* (2010)** observed inoculation of *Pseudomonas putida* GAP-P45 improved plant biomass, relative water content and leaf water potential by accumulation of proline in

maize plants exposed to drought stress. Proline concentration increased when maize plants were inoculated with *P. fluorescens* under drought stress **(Ansary *et al.,* 2012).** Drought tolerance of *Lavandula dentate* showed that PGPR strain *B. thuringiensis* (Bt) inoculation enhanced shoot proline accumulation when compared to control plants under drought stress **(Armada *et al*., 2014). Shintu and Jayaram, (2015)** reported that tomato (*Lycopersicon esculentum* Mill) when treated with phosphate solubilizing bacteria (*Bacillus polymyxa*) under drought conditions able to secrete excess proline.

2.4.11 Antioxidant metabolism

Levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants **(Mittler, 2002).** Several studies have demonstrated that generation of reactive oxygen species (ROS), such as the superoxide radical (O^{2-}), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) as a consequence of drought stress. ROS negatively affects the metabolic rate of plants as it causes oxidative damage to biomolecules such as lipids and proteins which eventually leads to cell death. To protect against oxidative stress plant cells produce enzymatic antioxidant such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), and non-enzymatic antioxidants such as ascorbate peroxidase (APX), glutathione reductase (POX) and tocopherol **(Mittler, 2002; Del Rio *et al*., 2003).** Levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants. **Stefan *et al.* (2013)** investigated antioxidant enzymes were increased in the presence of PGPR strains. SOD activity and peroxidase activity was significantly higher in the leaves of PGPR inoculated plants after 20 days of inoculation, compared with the control. **Gururani *et al*. (2013)** reported that inoculation of potato plants with PGPR (*Bacillus firmus* and *Bacillus pumulis*) induce the level of catalase and peroxides. **Saravana Kumar *et al.* (2011)** observed that green gram inoculated with *Bacillus subtilis* EPB and *Pseudomonas fluorescens* Pf1 results in increase the catalase enzyme activity.

2.5 Microbial consortium

A microbial consortium can be defined as “two or more microbial groups from different species live synergistically and carry out specific function in nature” (**Nain *et al*., 2010**). Consortium can be endosymbiotic and ectosymbiotic, they can be employ

for different useful activities such as metal transformation in metal contaminated soil, degradation of environment pollutant and stimulation of plant growth. Applications of potential microorganisms in a consortium enhancer reliability, consistency and efficacy of the microorganisms under different soil and environmental conditions **(Stockwell *et al.*, 2011).** For developing a microbial consortium microorganism should be a) nonpathogenic b) have enzymatic activity c) fast acting and synergistically active d) good sustainability e) resistant to environmental stress f) non corrosive of consistent quality and economical **(Choure and Dubey, 2012).**

2.6 Microbial consortium as PGPR

Microbial consortium is a unique strategy which can overcome the problem of growth and results in enhancement in yield of various crop systems under drought stress. Microbial consortium is a group of several microorganisms (frequently two) in which each organism contributes benefit to other organisms. Many fundamental processes in nature are the outcome of such interactions among microorganisms influencing the biosphere on a worldwide scale. Various microbial consortia are reported to enhance micronutrient uptake, yield, plant height, number of tillers, and dry weight of plant and grain yield of wheat crop **(Khalid *et al.*, 2004; Rana *et al.*, 2012).** Numbers of workers had evaluated the effects of PGPR on crop growth **(Chiarini *et al.*, 1998)** which belong to various genera and can enhance plants growth when inoculated singly or together with other strains. **Kakar *et al.* (2016)** investigated that the consortium of *Bacillus amyloliquefaciens* Bk7 and *Brevibacillus laterosporus* B4 (BB) with rice plants leads to the increased growth and productivity as compared to uninoculated plants. Consortium has the ability to confer induced systemic tolerance (IST) to cold and drought stress and helps the plants to survive under adverse abiotic stress conditions**.** Various researchers observed that the plant co-inoculation with different bacterial species may enhance the tolerance towards abiotic stresses and improve plant growth and yield **(Hiscox and Israelstam 1979; Figueiredo *et al.*, 2008; Wang *et al.*, 2012). Castillo *et al.* (2013)** studied the comparative effect of single bacterial inoculation and co inoculation and improved plant growth, productivity and high relative water content was found in the treatment of plants with consortia at par with single bacteria inoculation, so they concluded that the consortia of different bacterial species can improve better drought tolerance as compare with single bacterial inoculation. Many researchers have

successfully developed the combinations of different PGPR strains that significantly increased the biomass and yield of many plant species **(Verma *et al*., 2010; Yadegari *et al*., 2010)**. **Wang *et al*. (2012)** described the significant effect of a consortium of three rhizobacterial strains (*B. cereus* AR156*, B. subtilis* SM21, and *Serratia* sp. XY21) that was able to induce systemic tolerance towards drought stress and growth stimulation in cucumber plants**. Figueiredo *et al*. (2008)** applied a mixture of *Paenibacillus polymyxa* and *Rhizobium tropici*to common bean (*Phaseolus vulgaris* L.) and found a significant increase in plant growth and yield under drought stress. **Rajasekar and Elango (2011)** tested plant growth promoting rhizobacteria i.e. *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* separately and in combination *on Withania somnifera* for two consecutive years (2009 and 2010) and found that the consortium showed significantly increased plant height, root length, and alkaloid content in *Withania somnifera.* **Gusain *et al.* (2015)** PGPR consortia containing *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81 and *Arthrobacter nitroguajacolicus* strain YB3*,* strain YB5 enhanced plant growth in Sahbhagi (drought tolerance) and IR-64 (drought sensitive) cultivars of rice (*Oryza sativa* L.), in severe drought stress both the consortia treated varieties showed higher proline in almost similar way, the attribute might differentiate the tolerant and sensitive varieties of rice suggesting the vital role of proline as an osmoregulatory solute in plants. Higher proline accumulation in inoculated treatment indicates higher tolerance to water stress **(Gusain *et al*., 2015).**

2.7 Bioformulation

Bioformulation term refers to, putting two or more than two compatible microorganisms together according to their properties for desired purposes. Generally, microorganisms used in bio formulation has shorter shelf life therefore to enhance the shelf life suitable, cheap carrier material such as sand, wheat bran, charcoal, sugarcane bagasse and talc etc. can be used, which can increase shelf life of bioformulation by the protection of associated microorganisms as well as their useful metabolites and products. Use of bioformulation as biofertilizers can improve nutrient availability and their supplies by means of various mechanisms such as nitrogen fixation, siderophore production and mineral solubilization for plants uptake. This method is admirable due to its ecofriendly nature, cost effectiveness, easiness and renewability **(Mishra *et al*., 2015).** Application of biofertilizers to seed, plant soil or plant surface induces microbial

activities such as siderophore production, mineral solubilization, phytohormone production, nitrogen fixation and extracellular volatiles compound for enhancement of nutrient availability for easy assimilation by plants **(Sharma *et al*., 2012; Kumar *et al*., 2015; Ahmad *et al*., 2016; Jha and Subramanian, 2016).** Chemical fertilizers has high cost, long persistence in nature and non-renewability, therefore biofertilizers are greater alternative over chemical fertilizers **(Bahadur *et al*., 2014; Maurya *et al*., 2014; Jat *et al*., 2015; Kumar *et al*., 2016).** Carrier used in bioformuation is a thing which is the delivery vehicle of associated microorganisms and they also enhances their viability by protecting it from environmental stress **(Chen *et al*., 2000; Shahraki *et al*., 2009; Ardakani *et al*., 2010; Jorjani *et al*., 2011).** It also increases the durability of microbial metabolites such as antibiotics, hormones and volatile compounds **(Heijnen *et al*., 1993).** A good carrier should therefore possess following properties:

(a) Excellent moisture absorption capacity

(b) Material should not be lump forming

(c) Easy sterilization

(d) Low price and easily available

(e) Good pH buffering capacity

(f) Ability to deliver the right number of viable cells in good physiological condition at the right time **(Trevors *et al*., 1992; Meena *et al*., 2015).**

Materials and Methods

3.1 Culture retrieval

Total 18 cultures were obtained from departmental culture collection, Department of Microbiology, G.B. Pant university of agriculture and Technology, Pantnagar, Uttarakhand, which was originally isolated from the rhizospheric soil of wheat, maize, rice and soybean **(Rawat, 2016** and **Kumar, 2015).** For culture retrieval, 100µl of aliquot was withdrawn from glycerol stock and inoculated in nutrient broth and incubated at 28±2°C for 24 hours. Afterwards the cultures was kept on slants containing nutrient agar medium at 4°Cin refrigerator for further studies.

3.2 Screening for drought tolerance

All bacterial culture were screened for drought tolerance in tryptic soy broth (TSB) medium supplemented with different concentration of poly ethylene glycol (PEG) i.e. ranging from 2-10% **(Michel and Kaufmann, 1973).** The log phase cultures were inoculated into this medium and incubated at 28±2°C for 24-72 hours under shaking Conditions (120rpm). The growth was estimated by measuring the optical density (O.D.) at 600 nm using a spectrophotometer.

3.3 Characterization of bacterial isolates for PGPR traits

All the drought tolerant bacterial cultures were further screened for various plant growth promoting (PGP) traits.

- Siderophore production
- ACC deaminase activity
- Zinc solubilization
- Phosphorus solubilization
- Exopolysaccharide (EPS) production
- Hydrogen cyanide (HCN) production
- Indole acetic acid (IAA) production
- Ammonia production

3.3.1 Screening of siderophore producing bacteria

All screened drought tolerant bacterial strains were subjected to screening for siderophores production for which universal Chrome Azurol S (CAS)-agar medium **(Schwyn and Neilands, 1987)**. A loopful culture of test bacterial isolates were spot inoculate on CAS agar medium containing CAS dye as an indicator and plates were incubated at 28±2°C for 72 hours. Formation of orange or yellow colored zone around the bacterial colony was considered as positive results for siderophore production.

3.3.2 Screening of zinc solubilizing bacteria

Zinc solubilizing capacity of bacterial isolates were examined by plate assay containing basal agar medium supplemented with two insoluble sources of zinc, zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$). The plates of basal agar medium were spot inoculated and incubated at 28±2°C for 7 days. Zinc solubilization efficiency (SE) was calculated as described by **Ramesh *et al.* (2014).**

Solubilization efficiency % = (diameter of halo zone / diameter of colony) × 100

3.3.3 Phosphate solubilizing bacteria

Phosphate solubilizing efficiency of the bacterial isolates were determined using Pikovaskya's agar medium containing 5g/l of tri calcium phosphate (TCP). All bacterial isolates were spot inoculated on petriplates containing pikovaskya's medium supplemented with TCP and plates were incubated for 3-4 days at 37±2°C. Positive cultures were screened by observing transparent halo zones around the bacterial colony and the diameter of zone was measured.

Solubilization efficiency % = (diameter of halo zone / diameter of colony) × 100

3.3.4 Qualitative assay of ACC deaminase activity

To determine the qualitative estimation of ACC deaminase activity, DF (Dworkin and Foster) salt minimal medium supplemented with ACC as sole nitrogen source were used **(Honma and Shimomura, 1978)**. The bacterial suspension was prepared by growing test bacterial isolates in TSB medium at $28\pm2^{\circ}$C for 24 h in shaking incubator (120 rpm). Bacterial cultures were spot inoculated on petri plates containing DF salt minimal media supplemented with 3mM ACC. Plates containing DF salt minimal media with di-ammonium sulphate {$(NH_4)_2SO_4$} (0.2%w/v) were served as positive control and plates containing only DF salt minimal medium served as negative control. After incubation at

28±2°C for 72 h, the results were analyzed by observing the growth of bacteria on treatment plate when compared with positive and negative control.

3.3.5 Exo-polysaccharide (EPS) production

EPS production was analyzed by the method of **Modi *et al.* (1989).** 10 ml of basal medium (with PEG and without PEG) was inoculated with test culture (100µl) and kept for incubation at 28±2°C for 5-6 days. After incubation culture suspension was collected and centrifuged at 10,000 rpm for 15 minutes and thrice volume of chilled acetone was added and incubated overnight at 4°C. EPS will be separated from the mixture in the form of a slimy precipitates. Precipitates were collected on a predried filter paper. Allow the precipitates to dry overnight at 50°C and weighed the dried filter paper after overnight drying. The increase in the weight of filter paper is the amount of EPS produced.

3.4. Development of microbial consortia

All the screened potential drought tolerant bacteria *viz*. SKR14, MRCII6, RRCII3, SRP9II1, RRCI5 and RRNII2 were selected to develop the microbial consortia on the basis of biocompatibility of the bacterial strains with each other by preparing a individual lawn along with 5 isolates were streaked in bacterial lawn on nutrient agar plates. Consortia of biocompatible strains were prepared by adding equal amount of calculated colony forming (CFU) unit of all the strains and named as consortia 1, consortia 2, consortia 3, consortia 4 and consortia 5.

3.5. Morphological characterization

3.5.1 Colony morphology

Each bacterial culture was streaked on nutrient agar plates and incubated at 28±2 C for 24 hours. After incubation, colony morphological characteristics were analyzed. Following criteria were used to check colony morphological characterization:

- Shape
- Size
- Pigmentation
- Margin
- Texture
- Elevation
- Surface

3.5.2 Cell morphology

Cell morphology of bacterial cultures was checked with gram's staining. This differential staining technique can be used to know whether the bacteria are gram positive or negative. To carry out gram's staining a loopful bacterial culture was put on glass slide and stained with gram's staining kit as per the procedure of bergey's manual of determinative bacteriology **(Bergey *et al.*, 1994).** After staining the slide was observed under 100x objective lens microscope with immersion oil and analyzed for gram's reaction, arrangement and shape of cell.

3.5.3 Endospore staining

A differential staining technique was used to differentiate bacterial endospores and vegetative cells by **Scheaffer-fulton** method. Endospores are formed by a few genera of bacteria, such as *Bacillus*. By forming endospores bacteria can survive in adverse environmental conditions. The malachite green dye was used as primary stain in the endospore staining procedure and drive into cells with heat. After rinse of slide, a counter stain i.e. safranin was used to stain the decolorized vegetative cells. After staining the vegetative cells will appear pink and the spores will appear green.

3.6 Biochemical characterization

3.6.1 Catalase test

To determine the catalase production the loopful bacterial culture was inoculated into tryptic soy broth and incubated at 28±2°C for 24 hours. One drop of 3% hydrogen peroxide (H_2O_2) solution was added in the tube, immediate formation of bubbles or effervescence indicated the positive result for catalase production.

3.6.2 Gelatin hydrolysis

To determine gelatin hydrolysis 100µl of test bacterial cultures was inoculated in gelatin medium test tubes was prepared, and incubated at 28 ±2°C for 48 hours **(Aneja, 2003)** After incubation the test tubes were kept at 4°C for 1to 2 hour, and after refrigeration liquefied tubes indicated the positive results **(Thomas *et al.*, 2012).**

3.6.3 Cellulose activity

To determine the cellulose enzyme production test bacterial culture was spot inoculated on plates containing glucose yeast peptone agar (GYP) medium supplemented

with 0.5% carboxy-methyl cellulose (CMC) and incubated for 72 hours at 28±2°C. After incubation 0.2% congo red dye was used for staining and 1M NaCl solution used for destaining and left for 15 minutes **(Hankin and Anagnostakis, 1975).** Appearance of yellow color zone around the bacterial growth indicated positive results.

3.6.4 Starch hydrolysis

The amylase production activity of the bacterial culture was determined by method out lined by **Kasana *et al.* (2008)**. Test bacterial isolates were spot inoculated in Glucose yeast peptone agar plates supplemented with 0.2% soluble starch and incubated at 28 ±2°C for 1-2 days. After incubation plates were flooded with iodine solution. Appearance of purple color zone around bacterial growth indicated positive results.

3.6.5 Casein hydrolysis

To determine casein hydrolysis test bacterial cultures were spot inoculated in skim agar medium and incubated for 24-48 hours at 28±2°C **(Kasana *et al*., 2008).** Formation of halo zone around bacterial growth indicated positive results.

3.6.6 Laccase activity

Laccase activity was determined by spot inoculation of test bacterial culture on nutrient agar plates supplemented with 0.01% Guaicol and incubated at 35°C for 1-4 days **(Kiiskinen *et al*., 2004).** Red- brick color around the bacterial colony indicated positive results.

3.6.7 Urease production

To examine urease enzyme production, a loopful test bacterial culture was inoculated in Christensen's urea broth and incubated at 28±2°C for 24 hours. Change in color of inoculated medium from yellow to dark pink or red indicated positive results.

3.6.8 Lipolytic activity

To examine lipase production test bacterial cultures were spot inoculated on the plates containing tween 80 and incubated at 28±2°C for 24 to 48 hours **(Francisco *et al*., 1998).** Appearance of opaque zone around the bacterial growth indicated positive result.

3.6.9 Pectinolytic activity

To determine pectinase enzyme production, loopful test bacterial cultures were spot inoculated in plates containing Pectin agar medium and incubated at 28±2°C for 72 hours **(Aneja, 2003).** After incubation the plates were flooded with iodine solution. Appearance of clear zone around the bacterial growth indicated positive results.

3.6.10 Hydrogen sulfide (H_2S) production

To examine hydrogen sulfide production, test bacterial culture was inoculated in SIM (*Sulfide, Indole, Motility*) broth medium at 28 ±2°C for 3-4 days. After incubation the black precipitate was formed in inoculated test tube, which indicates positive result for H_2S production.

3.7.11 IMVIC test

It is a standardized colorimetric identification analysis, generally containing four biochemical tests and eight carbohydrate (glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose, sucrose) utilization tests. All tests are based on substrate utilization and alteration in pH. IMVIC tests were carried out on IMVIC kits (**Hi-Media, India**). 50 µl of log phase aliquot of test organisms was inoculated in each well of IMVIC kit and incubated at 37±2°C for 24 h. All the tests performed according the directions given by manufacturers. IMVIC kit includes following tests-

1. **Indole test-** After incubation period, 1-2 drops of Kovac's reagent was added into the well. Change in color of the medium from yellow to reddish pink within 15 seconds considered as positive result.
2. **Methyl red test-** After incubation, 1-2 drops of Methyl red reagent was added into the well. The change in color of medium to red considered as positive result.
3. **Voge's proskeur test-** Following incubation, 1-2 drops of baritt reagent "A" and barritt reagent "B" were added sequentially. After 8 – 10 min, appearance of red color indicated as positive result.
4. **Citrate utilization test-** After incubation, color change from green to blue indicated positive reaction.
5. **Carbohydrate fermentation test-** After incubation period of 48 h, color of medium changed from red to yellow indicated positive results for sugar utilization.

3.7. Comparative study of PGPR traits between single bacterial and consortia

Plant growth promoting characteristic and functional traits of individual bacterial isolates and prepared consortium were further checked and analyzed comparatively through qualitative and quantitative estimation.

3.7.1. Quantitative estimation of siderophore production

Siderophore produced by individual isolates and all selected bacterial consortia were quantified using CAS-shuttle assay followed by the method of **Payne (1994)**. All cultures were grown in iron free minimal medium and incubated for 24 hours at 28±2°C and samples were withdrawn and centrifuged at 3000 rpm for 15 min. In 1.5 ml of culture supernatant 1.5 ml of CAS dye solution and 30µl of shuttling solution (sulfo-salicyclic acid) were added, mixed and allowed to stand for 20 min. If the siderophore present, it removes the iron from the dye complex, causing reduction in the intensity of blue color and the absorbance was recorded at 630nm. Minimal medium was used as blank and % siderophore units were calculated by following formula:

$$\% \text{ siderophore units} = [(A_r - A_s)/A_r] \times 100$$

Where absorbance of reference

A_r = (minimal media + CAS assay solution), A_s = absorbance of sample

3.7.2 Quantification of zinc solubilization by AAS

The test bacterial cultures and selected consortia were inoculated separately to basal medium supplemented with 0.1% insoluble zinc compounds (ZnO and $ZnCO_3$) and incubated at 28±2°C for 14 days. Samples were withdrawn and centrifuged at 8000 rpm for 10 min. 10ml supernatant was fed to Atomic Absorption Spectrophotometry (AAS) for determination of the available zinc content.

3.7.3 Quantification of phosphate solubilization

3.7.3.1 Stock solutions and reagents used

a. Stock solution of KH_2PO_4 (10mg/ml)

b. 2.5% Ammonium molybdate solution-2.5g of ammonium molybdate (NH4) $Mo_7O_27.H_2O$) was dissolved in (20ml) distilled water and 10 N H_2SO_4(50 ml) was added to it. The volume was makeup to 100ml with distilled water.

c. Coloring reagent (10 ml)-

i. 15% sodium bisulphide solution.

ii. 20% sodium sulphide solution.

iii. 25 mg 1-amino-2-napthol 4-sulphonic acid (ANS).

250 µL of solution (ii) was added to the 250µl of solution (i), then 25 mg ANS was mixed and filtered through whatman No. 1 filter paper and final volume was made up to 10 ml with distilled water.

d. 60% perchloric acid (PCA) solution.

3.7.3.2 Standard curve

To prepare the standard curve 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 µL aliquots of KH_2PO_4 stock solution were taken separately in test tubes and volume is made up to 1ml by adding distilled water. It was followed by addition of 2.5% of ammonium molybdate (400 µL) solution and coloring reagent, ANS (200 µL). Final volume was made up to 5ml by adding 3.4ml of distilled water. The tubes were then incubated at room temperature for 10 min. The absorbance was recorded at 640 nm and standard curve (O.D v/s concentration) was drawn. Distilled water (5ml) was taken as control.

3.7.3.3 Quantitative P solubilization

Active bacterial cultures were inoculated separately in NBRIP broth and incubated at 28±2ºC (120 rpm) for nine days. Samples were withdrawn periodically at 3rd, 5th, 7th, 9th day and centrifuged at 5,000 rpm (4ºC) and supernatant was used as culture filtrate for analysis. 400 µL of PCA solution was added followed by ammonium molybdate solution (400 µL) and ANS coloring reagent (200 µL) was added to supernatant (1ml). The final volume was made up to 6mL with distilled water in each tube and incubated for 10 minutes at room temperature. The absorbance was recorded at 640nm and values were determined with respect to the standard curve of phosphate.

3.7.4 Qualitative and quantitative estimation of IAA production

The production of indole acetic acid (IAA) by bacterial cultures and consortia were assayed by the method of **Gordon and Weber (1951).** For this the bacterial

cultures were grown on 10ml luria broth amended with 100μg ml^{-1} tryptophan and incubated at 28±1˚C for 2-4 days. Bacterial suspension was centrifuged at 10,000 rpm for 5 min and supernatant was collected. In 2ml of supernatant 4ml of salkowski reagent was added and allow to stand at room temperature for 30 minutes. Appearance of pink color was served as positive result, optical density was measured by spectrophotometer at 530 nm and the amount of IAA was obtained by standard curve. The intensity of pink color was read at 530 nm spectrophotometrically and the amount of IAA produced was extrapolated from the standard curve.

Standard Curve

IAA stock solution -100 μg/ml standard stock solution of IAA in 50% ethanol was prepared.

Preparation of the standard curve

Standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 ml of standard IAA solution in test tubes. The volume was made to 2 ml with distilled water and then 4 ml of Salkowski reagent was added and tubes were incubated for 25 minutes at room temperature. After incubation optical density was measured at 530 nm. Standard curve was prepared by plotting absorbance at 530 nm against concentration of IAA solution.

3.7.6 Ammonia production

Determination of ammonia production was done by the method given by **Bakker and Schippers (1987).** All test cultures and consortia were grown in peptone water for 2-3 days at 28± 2°C. After incubation, Nessler's reagent (1ml) was added in each tube. Appearance of faint yellow color indicated small amount of ammonia whereas brownish color indicates high amount of ammonia.

3.7.7 Hydrogen cyanide (HCN) production

Production of hydrocyanic acid (HCN) was detected by the method of **Castric (1974)** on nutrient agar plates supplemented with 4.4g/l glycine streaked with the test bacterial isolates. Filter paper strips dipped in picric acid and 2% sodium carbonate (Na_2CO_3) were placed in the lids of each petri plates and plates were sealed with paraflim, incubated at 28±2°C for 3-4 days. HCN production was checked on the basis

of changes in colour of filter paper strip from yellow to light brown, moderate brown or strong brown of the yellow filter paper strips.

3.7.7 *In vitro* antifungal activity assay

The antifungal activity of bacterial cultures and consortia were analyzed against *Fusarium* and *Sclerctonia* was determined through dual culture method. Briefly, a mycelial disc was cut from the white perimeter of a colony that had been cultured on half strength PDA plus NA plates at 25±2°C for seven days. The disc was placed 1.5 cm from the edge of a fresh PDA plate, and then a sterilized toothpick was used for spot inoculation of test bacterial cultures into the same plate, 1.5 cm from the opposite edge. Control plates without microorganisms were prepared simultaneously. The antagonistic effect was determined by measuring the size of the inhibition zone formed on plates after incubation at 25±2°C for 14 days.

3.8 Antibiotic sensitivity test

Antibiotic sensitivity behavior of the test bacterial isolates was determined by the disc diffusion method **(Bauer *et al*., 1966).** Antibiotic discs [Tetracycline (TE^{30}:3oμg/disc) ampicillin (AMP^{10}: 10μg/disc), Gentamicin (G^{10}:10μg/disc), Chloramphenicol (C^{50}:50μg/disc), Streptomycin (S^{25}:25μg /disc), Penicillin-G (P^{10}:10μg/disc)] of known potency were procured from hi media lab, Mumbai, India. Active culture of test bacteria culture was prepared and 100μl culture was placed on nutrient agar plate and spread evenly with spreader. Plates were mounted with antibiotic disc and incubated at 28±2̊ C for 24 hours. After incubation zone of inhibition was measured around each antibiotic disc. Plates were scored for resistance or sensitivity by comparing the chart based on the diameter of zone of inhibition as provided by the manufactures.

3.9 *In situ* assessment of potential bacterial consortium on wheat in drought stress under greenhouse conditions

Potential bacterial consortia were examined for their plant growth promontory activity under drought stress condition by performing *in situ* pot experiment in green house at Department of Microbiology, College of Basic Sciences and Humanities, GBPUAT Pantnagar, Uttarakhand India. The experiment was performed in three replicates in wheat crop (*Triticum aestivum* var. PBW373) experimental layout is given below:

Table 3.1: Particulars of the pot experiment

S. No.	Particulars of layout plan	Number/ size
1	Total numbers of treatments	7
2	Number of replications per treatment	3
3	Total numbers of pots	21
4	Capacity of each pot	5Kg
5	Soil used in each pot	4Kg
6	Experimental design	CRD

Table 3.2: Treatments used in the pot experiment

S. No	Treatments	Bacterial consortia
1	Control 1	Positive control
2	Control 2	Negative control
3	Bacterial consortium 1	SRK14, MRCII6
4	Bacterial consortium 2	SRP 9II1 , RRCI5
5	Bacterial consortium 3	RRNII2, SRK14, SRP 9 II 1
6	Bacterial consortium 4	RRCII3, SRP9II1
7	Bacterial consortium 5	RRNII2, RRCI5

3.9.1 Soil collection and preparation of experimental pots

Soil for pot experiment was collected from Crop Research Centre (CRC), GBPUA & T, Pantnagar (29° 01' 20.00" N, 79° 29' 15.00" E). Then soil were air dried with regular mixing to maintain the water content become > 1%. Later, soil was transferred in a muslin cloth bag and autoclaved for 3 times in three continuous days. This autoclaved soil mixed with 2% of FYM (Farmyard manure) and transferred to the pot (4Kg/ pot).

3.9.2 Seed selection

For pot trial wheat (*Triticum aestivum* var. PBW373) seeds were procured from C.R.C., G.B.P.U.A. & T., Pantnagar.

3.9.3 Seed sterilization

Seeds were surface sterilized before sowing. First seeds were rinsed with sodium hypochloride for 3 min followed by rinse with distilled water and treated with 95% ethanol for 1 min. Then seeds were washed with 0.1% $HgCl_2$ for 3 min and finally further washed eight times with sterilized distilled water. All these steps were performed under aseptic conditions **(Etesami *et al.,* 2009).**

3.9.4 Preparation of inoculum

Selected bacterial consortia were prepared by adding equal amount of respective bacterial culture (OD_{600nm} = 1.0), inoculated in 50ml nutrient broth and incubated for 24 hours at 28±2°C.

3.9.5 Seed bacterization

Ten seeds were dipped into each bacterial suspension (10^8cfu/ml) and incubated for 4-5 hours at 28±2°C. Seeds without bacterial culture served as control.

3.9.6 Sowing of seeds

Seeds treated with test bacterial cultures were sown on 23th November, 2017. Ten seeds were sown in each pot. After 21 days of emergence, all treatments were subjected to drought stress by discontinuous watering to pots. The positive and negative control were also maintained, the positive control was without bacterial culture under continuous watering served as positive control and pots with discontinuous watering and without bacterial inoculums served as negative control

3.9.7 Variety used

In the pot experiment early variety of wheat (*Triticum aestivum*) var. PBW373 was used.

3.8 Sample collection and biochemical analysis of leaves

Leaves sample from each treatment was collected in various intervals during their whole life cycle and biochemical analysis was done in collected leaves sample.

3.10.1 Relative water content

RWC (Relative water content) of leaves was measured at the time of each sampling at various intervals by the method of **Weatherley (1950).** weight of the sample leaves was measured and leaves were dipped into distilled water for 3-4 hours and weight of fully turgid leaves were recorded. Afterward, the leaves were dried in hot air oven at 70 °C for 72 hours and afterwards weight were recorded. Relative water content was measured by using following formula.

% RWC = (FW – DW / FTW–DW) x 100

Where: RWC = relative water content, DW = dry weight,

FW = fresh weight, FTW =fully turgid weight.

3.10.2 Total Chlorophyll content

Chlorophyll content was determined by the method of **Hiscox and Israelstam (1979).** 50 mg of leaf sample was taken and chopped into tiny pieces and transferred into test tubes containing 10ml Di-methyl sulphoxide (DMSO). Then the test tubes were kept into water bath for 3 hours at 60°C. Afterwards, absorbance was measured at 663 nm and 645 nm in dark. Chlorophyll content was calculated by the following formula.

$$\text{Chlorophyll a} = \frac{[12.7\text{x } A_{663} - 2.69 \text{ x } A_{645}] \text{ x V}}{1000 \text{ x W}}$$

$$\text{Chlorophyll b} = \frac{[22.9\text{x } A_{645} - 4.68 \text{ x } A_{663}] \text{ x V}}{1000 \text{ x W}}$$

$$\text{Total chlorophyll} = \frac{[20.2\text{x } A_{645} + 8.02 \text{ x } A_{663}] \text{ x V}}{1000 \text{ x W}}$$

Where: A = Absorbance at specific wave length,

V = Final volume of solution

W = fresh weight of tissue

3.10.3 Estimation of carotenoid content

Carotenoid content in leaf was determined by the method described by **Krik and Allen (1965).** Chopped leaves sample was suspended in Di-methyl sulphoxide (DMSO) and after 3 hour incubation at 60ºC, optical density was measured at 480nm.

$$\text{Carotenoids } (\mu g/g.fr.wt.) = A480 + (0.114 \times A663) - (0.638 \times A\ 645)$$

3.10.4 Proline content

Proline content was determined at the time of each sampling by method described by **Kandpal *et al.* (1981).** Leaf extract was prepared by grinding 250 mg of fresh leaf sample in 3% sulpho-salicylic acid. Then leaf extract were centrifuged at 3000 rpm for 10 minutes and 2ml aliquot of supernatant was dispensed in fresh test tube. 2ml aliquot of both the solution acid ninhydrin and glacial acetic acid was added. Afterwards, test tubes were incubated at 100°C in water bath for 1hour and reaction was terminated in ice bath, and then 4ml of toluene was added. Development of pink color indicated presence of proline. To estimate the proline level, the absorbance of acquired upper pink phase was measured at 520 nm.

Stock solution of proline

Stock solution of proline (1mg/ml) was prepared in distilled water.

Standard curve preparation

To prepare the standard curve 10, 20, 40, 60, 80 and 100µl aliquots of stock solution taken in fresh test tubes and volume was make up to 2 ml. 2ml of glacial acetic acid and 2ml of acid ninhydrin were added in each test tube. The test suspension incubated in water bath at 100°C for 1h and reaction was terminated in ice bath. After addition of 4ml toluene, absorbance was measured at 520nm.

3.10.5 Catalase activity

Catalase activity in leaf was determined by the method described by **Luck (1974).** To measure the catalase activity, 100 µl of enzyme extract was added to 3ml of H_2O_2 phosphate buffer (pH 7) and measured optical density at 240nm and time required for change in absorbance by 0.05 units was noted as ΔT. Enzyme solution containing only H_2O_2 served as control. Finally activity of catalase was calculated by following formula:

$$\text{Units in the assay mixture} = \frac{17}{\Delta T}$$

3.10.6 Lipid peroxidation

The lipid peroxidation activity was determined by the method described by **Health and packer (1968)**. The fresh 0.1gm of leaves was homogenize by adding 1ml of 0.1 % (w/v) TCA and centrifuged for 10 min at 15000g. 0.5 ml of supernatant added with 1.5 ml of 0.5% TBA diluted in 20% TCA and incubated at 90°C for 25 minutes. The reaction was ended by incubating on ice. The absorbance was measured at 532 and 600 nm.

3.11 Harvesting of wheat

All the wheat plants were harvested on 4 March 2018. All harvested plants were air dried and weighed in grams.

3.11.1 Plant height

To determine the plants height, root length and shoot length were measured.

3.11.1.1 Root length

Root lengths of all four plants were measured from base of stem to end of root tip and mean values were recorded in cm.

3.11.1.2 Shoot length

Shoot lengths of plants were measured from base of stem to longest spike and mean values were recorded in cm.

3.11.2 Plant dry weight

Randomly four plants were selected and individually all plant's weight were recorded. And the average weight of plant's expressed in gram.

3.12 Evaluation of shelf life of bacterial consortium

3.12.1.1 Preparation of talc-based formulation

The talc-based formulations of the bacterial consortia were prepared by following the method described by **Vidhyasekaran and Muthamilan (1995).** A loopful of bacteria was inoculated into the nutrient broth and incubated in a rotary shaker at 150 rpm for 48 h at room temperature (25 ± 2°C). Talc powder was taken and its pH was adjusted to neutral by adding $CaCO_3$ at the rate of 15 g/kg and 10 g of CMC was added to 1 kg of talc and mixed well then the mixture was autoclaved two consecutive days for 30 min. 10ml of 24 h grown bacterial consortia suspension was mixed with carrier-cellulose mixture (50gm in each packet) under aseptic conditions

and packed in polypropylene bag, sealed and stored at room temperature (25±2°C), in refrigerator at 4°C and outdoor conditions.

3.12.1.2 Preparation of sugarcane bagasse based formulation

Sugarcane bagasse was dried and grind in powdered form and autoclaved for 30 min on each of two consecutive days. The 5ml of 48 h grown bacterial consortia suspension was mixed with sugarcane bagasse (20gm in each packet) under aseptic conditions and packed in polypropylene bag, sealed and stored at room temperature (25 ± 2°C), refrigerator at 4°C and outdoor condition.

3.12.2 Shelf life detection

After every 15 day time interval samples were checked for measuring colony forming units (CFU) up to 90 days. Average cell number was calculated by estimating CFU/g of formulation on nutrient agar medium.

Table 3.3: Prepared bio-formulations and storage conditions

A) Talc based

S.N.	Formulation name	Bioformulation description (ingredients)
1	TL1	Talc + consortia 1 stored at 4°C
2	TL2	Talc + consortia 2 stored at 4°C
3	TL3	Talc + consortia 3 stored at 4°C
4	TL4	Talc + consortia 4 stored at 4°C
5	TL5	Talc + consortia 5 stored at 4°C
6	TR1	Talc + consortia 1 stored at room temperature
7	TR2	Talc + consortia 2 stored at room temperature
8	TR3	Talc + consortia 3 stored at room temperature
9	TR4	Talc + consortia 4 stored at room temperature
10	TR5	Talc + consortia 5 stored at room temperature
11	TO1	Talc + consortia 1 stored outside
12	TO2	Talc + consortia 2 stored outside
13	TO3	Talc + consortia 3 stored outside
14	TO4	Talc + consortia 4 stored outside
15	TO5	Talc + consortia 5 stored outside

B) Sugarcane bagasse based

S.N.	Formulation name	Bio formulation description (ingredients)
1	BL1	Sugarcane bagasse + consortia 1 stored at 4°C
2	BL2	Sugarcane bagasse + consortia 2 stored at 4°C
3	BL3	Sugarcane bagasse + consortia 3 stored at 4°C
4	BL4	Sugarcane bagasse + consortia 4 stored at 4°C
5	BL5	Sugarcane bagasse + consortia 5 stored at 4°C
6	BR1	Sugarcane bagasse + consortia 1 stored at room temperature
7	BR2	Sugarcane bagasse + consortia 2 stored at room temperature
8	BR3	Sugarcane bagasse + consortia 3 stored at room temperature
9	BR4	Sugarcane bagasse + consortia 4 stored at room temperature
10	BR5	Sugarcane bagasse + consortia 5 stored at room temperature
11	BO1	Sugarcane bagasse + consortia 1 stored outside
12	BO2	Sugarcane bagasse + consortia 2 stored outside
13	BO3	Sugarcane bagasse + consortia 3 stored outside
14	BO4	Sugarcane bagasse + consortia 4 stored outside
15	BO5	Sugarcane bagasse + consortia 5 stored outside

3.13 Statistical analysis

Under statistical analysis, programme named completely randomized design (CRD) which uses STPR3 determine the variability in data if each treatment. The programme was acquired from Department of Mathematics, Statistics and Computer Science, G.B.P.U.A.T, Pantnagar, Uttarakhand.

Results and Discussion

4.1. Retrieval of bacterial isolates

In the present study, 18 bacterial isolates were retrieved from Departmental culture collection, Department of Microbiology, *College of Basic Sciences and Humanities,* G.B.P.U.A&T, Pantnagar, Uttarakhand, India. It has been reported earlier that adaptation of microorganisms can play a major role in determining the plant growth promoting rates (**Kumar, 2015; Rawat, 2016; Khan, 2017**). Therefore, considering the fact, bacterial isolates were originally isolated from the rhizospheric soil samples of soybean, maize and rice, earlier characterized by **Rawat (2016)** and **Kumar (2015).** All bacterial isolates were further tested for drought tolerance and other plant growth promoting properties.

4.2. Screening of bacterial isolates for drought resistance

Out of 18 bacterial isolates, 9 isolates were showing highest growth (O.D.>0.6-0.7) at maximum 10% PEG 6000 (Poly Ethylene Glycol 6000) which causes osmotic stress **(Table 4.1).** It was shown that Poly Ethylene Glycol didn't showed any negative effect on the growth profile of bacteria, as stationary phase was achieved within 3 days itself and bacterial biomass was comparatively higher. Similar kind of results were also shown in the previous study conducted by **Michel and Kaufmann, (1973); Sandhya *et at.* (2009); Hamayun *et al.* (2010)**; **Susilowati *et al*. (2018).** The bacterial isolates showed turbidity in nutrient broth supplemented with PEG was further selected for determining their plant growth promoting activities.

Table 4.1: List of drought tolerant bacterial isolate and their site of isolation

S.N	Description of bacterial isolates	Isolation site	References
1	SRM7, SRM11	Soybean rhizosphere, Mavakot, Uttarakhand	**Rawat (2016)**
2	SRK14	Soybean rhizosphere, Kotdwar, Uttarakhand	**Rawat (2016)**
3	SRPII7, SRP9II1	Soybean rhizosphere, Pithoragarh, Uttarakhand	**Rawat (2016)**
4	RRCI5, RRNII2, RRCII3	Rice rhizosphere, Crop Research Centre (CRC), Pantnagar, Uttarakhand	**Kumar (2015)**
5	MRCII6	Maize rhizosphere, Crop Research Centre (CRC), Pantnagar, Uttarakhand	**Kumar (2015)**

4.3. Screening of drought tolerant bacteria for plant growth promoting traits

Plant growth promoting rhizobacteria can contribute to alleviate abiotic stresses of staple crops via a variety of mechanisms. Lowering of ethylene level by 1-aminocyclopropane-1-carboxylate (ACC) deaminase is considered to be one of the major mechanisms employed by PGPB to favor plant growth under stress conditions. Bacterial exopolysaccharide (EPS), protects microorganisms from drought stress due to its water retention ability and regulates the diffusion of organic carbon sources **(Whipps *et al*., 1990).** Other metabolites produced by PGPB are potentially involved in supporting plant growth under stress conditions include siderophores, organic acids and antioxidant enzymes. All drought tolerant bacterial isolates were further tested for plant growth promoting traits such as siderophore production, zinc solubilization, phosphate solubilization, ACC deaminase activity and EPS production.

Among nine drought tolerant bacterial isolates RRCI5, MRCII6, RRNII2, RRCII3, SRK14 and SRP9II1 showed higher efficiency for all tested PGPR traits **(Table 4.2).** Six were positive for siderophore production, seven were positive for zinc and phosphate solubilization, five were positive for ACC deaminase activity and all nine bacterial isolates positive for EPS production. EPS production is a common trait of PGPR and it's a property of rhizospheric bacteria. In the present study after incubation in tryptic soy broth media for 5 days, the maximum amount of EPS production was observed in isolate RRCII3 i.e. 15.2 mg/ml under drought stress conditions **(Table 4.2).** However, remaining bacterial isolates produced good amount of EPS in the range of 10.6 mg/ml to 14.6 mg/ml. Under non stress conditions RRNII2 produces highest amount of EPS i.e. 10.2 mg/ml. it was observed that under stress conditions the bacterial isolates produced more EPS as compare to the non-stress conditions. As EPS are known to be useful to improve the moisture-holding capacity which may be good candidate to develop bioinoculant for drought conditions. **Vardharajula and sKz** (**2014)** reported that the increase in EPS production in three *Bacillus* sp. strains increased with increasing water stress, indicating correlation between drought stress tolerance and EPS production. On the basis of efficiency of plant growth promoting traits final six bacterial culture *viz.* RRCI5, MRCII6, RRNII2, RRCII3, SRK14 and SRP9II1 were selected further for consortia development and their morphological and biochemical characterization.

Table 4.2: Plant growth promoting properties of selected potential bacterial isolates

S.N.	Bacterial isolates	Siderophore production	Phosphate solubilization	Zinc solubilization	ACC deaminase	EPS production mg/ml	
						With PEG	Without PEG
1	SRM 7	-	++	+	-	10.6	7.2
2	SRM 11	-	-	-	-	10.0	7.2
3	SRK 14	++	++	++	-	12.4	8.4
4	SRP II 7	++	-	-	-	11.2	9.6
5	SRP9 II 1	-	+++	+++	+++	10.8	7.6
6	RRC I 5	+++	+++	+++	+++	14.6	9.0
7	MRC II 6	+++	+++	+++	+++	12.2	7.8
8	RRCII3	+++	+++	+++	+++	12.6	10.2
9	RRNII2	+++	+++	+++	+++	15.2	9.4

+++ = Highest efficiency, ++ = Moderate efficiency, + = Low efficiency, - = no efficiency

4.4. Consortia preparation

The six best potential bacterial strains *viz*. SRPII9I, SRK14, MRCII6, RRNII2, RRCI5 and RRCII3 were selected for the development of microbial consortia on the basis of biocompatibility of strains with each other **(Figure 4.1)**. The consortium was prepared by adding equal amount of respective bacterial culture (OD_{600nm} = 1.0) and used for further plant growth promontory studies. Total five consortia were developed and named Consortia 1, Consortia 2, Consortia 3, Consortia 4 and Consortia 5, mentioned in the **Table 4.3.** Since, the employment of microbial consortium offers considerable advantage over the use of pure cultures in plant growth promotion considering its multifunctional ability to fulfill the task in an efficient way than single-strain inoculation and can be more robust to environmental changes **(Guetsky *et al.*, 2002; Shenoy and Kalagudi, 2003).** So in the present study, we analyze the comparative capabilities of consortia and single strains in plant growth promotion under drought stress.

Table 4.3: List of bacterial consortia and their respective bacteria

S.N.	Laboratory name for the consortia	Bacterial isolates
1	Consortia 1	SRK14, MRCII6
2	Consortia 2	SRP9 II 1, RRCI5
3	Consortia 3	RRNII2, SRK14, SRP9II1
4	Consortia 4	RRCII3, SRP9II1
5	Consortia 5	RRNII2, RRCI5

4.5. Morphological characterization of the selected bacteria

All six selected bacterial isolates which were used in the preparation of bacterial consortia were morphologically characterized and summarized in **Table 4.4.**

Table 4.4: Cultural characteristics of the screened bacterial isolates.

a) Colony morphology

S.N.	Bacterial isolates	Colony characteristics				
		Shape	Edge	Elevation	Surface	Chromogenesis
1	SRP9 II 1	Circular	Entire	Raised	Smooth	White
2	RRC I 5	Circular	Entire	Convex	Shiny	Creamy
3	MRC II 6	Circular	Entire	Convex	Smooth	Pale yellow
4	RRN II2	Circular	Entire	Raised	Smooth	White
5	RRCII3	Irregular	Undulate	Raised	Shiny	Creamy
6	SRK14	Circular	Entire	Flat	Smooth	Pale yellow

b) Cell morphology

S.N.	Bacterial isolates	Gram reaction	Cell morphology	Arrangement
1	SRP9 II 1	Negative	Short rods	Scattered
2	RRC I 5	Negative	Short rods	Scattered
3	MRC II 6	Negative	Short rods	Scattered
4	RRN II2	Negative	Short rods	Scattered
5	RRCII3	Negative	Short rods	Chain
6	SRK14	Negative	Short rods	Scattered

4.6. Biochemical characterization of the screened bacteria

4.6.1. Production of extracellular enzyme

Screened and selected potential bacterial cultures were further characterized for production of extracellular enzymes. Recent studies on hydrolytic enzymes showed their ability to control plant pathogens (**Shaikh and Sayyed, 2015; Shaikh *et al.,* 2016**). Hydrolytic enzymes like chitinase, glucanase, protease and cellulase are able to degrade the fungal cell-wall and cause the cell lysis of fungal pathogens.

Extracellular enzyme production assay performed in the present study confirmed that only isolates SRP9II1 was positive for amylase production **(Table 4.5.1; Figure 4.2 a).** SRP9II1, RRCI5, MRCII6, RRNII2 were positive for gelatinase **(Table 4.5.1; Figure 4.2 b).** SRP9II1, MRCII6, RRNII2 were positive for ceasinase **(Table 4.5.1; Figure 4.2 c)**.Only SRK14 positive for H_2S production **(Table 4.5.1; Figure 4.2 d).** No bacterial isolates were found to be positive for pectinase and laccase enzyme activity. RRCI5, RRNII2 and SRK14 were positive for cellulose **(Table 4.5.1; Figure 4.2 e).** Urease was produced by SRP9II1, RRNII2, MRCII6 and SRK14 **(Table 4.5.1; Figure 4.2 f)**. SRP9II1, MRCII6, SRK14 and RRNII2 were positive for lipase activity **(Table 4.5.1; Figure 4.2 g)**. Whereas, all bacterial isolates were positive for catalase production **(Table 4.5.1; Figure 4.1 h)**.

4.6.2. IMVIC

All selected bacterial isolates were examined for indole, methyl red, voge's-proskauer, citrate utilization test through IMVIC Kit (Himedia, India) summarized in **(Table 4.5.2; Figure 4.2 i).** Out of six bacterial isolates, SRK14 was positive for indole test, SRP9II1, RRCI5, RRNII2 and SRK14 were positive for methyl red test. SRP9II1, RRCII3 and SRK14 bacterial isolates were positive for voge's proskauer test, SRP9II1, RRCI5, RRNII2, RRCII3 and SRK14 were positive for citrate utilization. All bacterial isolates were also tested to metabolize various sugars. SRK14 was able to utilize glucose whereas all other bacterial isolates were able to metabolize wide range of sugars via production of various enzymes **(Table 4.6; Figure 4.2 i).**

Table 4.5.1: Biochemical characteristics of selected potential bacterial isolates

S.N	Bacterial isolates	Amylase	Gelatin	Catalase	Pectinase	Laccase	Cellulose	H_2S	Urease	Lipase	Ceasinase
1	SRP9 II 1	+	+	+	-	-	-	-	+	+	+
2	RRC I 5	-	+	+	-	-	+	-	-	-	-
3	MRC II 6	-	+	+	-	-	-	-	+	+	+
4	RRN II2	-	+	+	-	-	+	-	+	+	+
5	RRCII3	-	-	+	-	-	-	-	-	-	-
6	SRK14	-	-	+	-	-	+	+	+	+	-

Table 4.5.2: IMVIC test of selected potential bacterial isolates by using Hi-IMVIC Kit

S.N	Bacterial isolate	Indole	Methyl red	Voge's proskauer	Citrate	Glucose	Adonitol	Arabinose	Lactose	Sorbitol	Mannitol	Rhamnose	Sucrose
1	SRP9II1	-	+	+	+	+	+	+	-	+	-	_	+
2	RRC I 5	-	+	-	+	-	-	+	+	+	+	+	-
3	MRCII6	-	-	-	-	+	+	+	-	+	+	+	+
4	RRN II2	-	+	-	+	+	-	+	+	+	+	+	+
5	RRCII3	-	-	+	+	+	+	+	+	+	+	+	+
6	SRK14	+	+	+	+	+	_	_	_	_	_	_	_

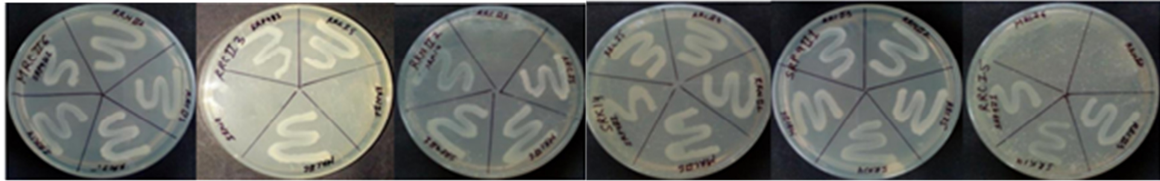


Fig 4.1: Compatibility assay

(a) (b)

(c) (d)

(e) (f)

(g) (h)

(i)

Fig 4.2: Results of biochemical characterization

a) Amylase production, b) Gelatinase, c) Casinase, d) Urease,
e) Cellulase, f) H_2S, g) Lipase, h) Catalase and i) IMVIC test

4.7. Characterization of bacterial isolates and consortia for PGPR traits

4.7.1 Qualitative assay of siderophore production

All selected bacterial isolates and consortia incubated on CAS (Chrome azurol S) agar medium. All positive siderophore isolates showed different zone diameter which defines the different level of siderophore production. The CAS dye chelates ferric form of iron by complex formation and appear as color change form blue to orange halo zone. Therefore, a result of siderophore production was interpreted on the basis of the size of the orange halo zone around the bacterial colonies presented in **Table 4.6; Figure 4.3**. However, the siderophore production efficiency of single bacterial and consortia were almost in the same range, Out of which RRCI5 showed highest efficiency i.e. 188.8% followed by consortia 4 which showed 157.7% siderophores production efficiency. **Saisirekha and Srividya (2016)**, detected siderophore production using CAS agar which showed orange-coloured colonies after 24 h of incubation due to the siderophoral removal of Fe from the dye which primarily indicated the ability of *P. aeruginosa* FP6 to produce siderophore.

Table 4.6: Iron solubilization potential of bacterial isolates and microbial consortium on CAS agar medium

S.N.	Bacterial isolates	Iron chelation		
		Colony diameter (cm)	Clearing Zone (cm)	Solubilization efficiency (%)
1	SRP9 II 1	0.9	1.4	155.5
2	RRC I 5	0.9	1.7	188.8
3	MRC II 6	1.0	1.1	110
4	RRN II2	0.8	1.3	162.5
5	RRCII3	0.6	0.9	150
6	SRK14	1.1	1.0	90.9
7	Consortia 1	1.9	2.2	115.7
8	Consortia 2	1.8	2.4	133.3
9	Consortia 3	2.0	2.4	120
10	Consortia 4	1.9	2.2	157.7
11	Consortia 5	1.7	2.1	123.5

4.7.2. Quantification of solubilized iron

After evaluating the efficiency of siderophore production by bacterial isolates and consortia qualitatively, the same were further analyzed for quantitative estimation of iron chelation efficiency through spectrophotometric analysis **(Table 4.7).** Consortia 1 showed highest 67.88% siderophore unit whereas individual isolate RRNII2 showed maximum **54.45**% siderophore unit in single culture. Efficiency of siderophore production observe to be more in the consortia as compare to the single bacterial isolates i.e. ranged from 54.63% to 67.88% and 41.39% to 54.45%, respectively **(Table 4.7).**

Similar results were reported by **Jha and Saraf (2012),** where siderophore production under the mixed coinoculation of *B. Brevis* (MS1)*, B. licheniformis* (MS3) and *A. calcoaceticus* (MS5), was 67.59 L g/ml after 96 h. whereas MS5 and MS53 alone produced the maximum amount of siderophore, that is, 32L g/ml and 22L g/ml, respectively after 96 h. Siderophore production of MS3 increased by the mutualistic association with MS5 during coinoculation.

Table 4.7: Quantitative analysis of siderophore produced by bacterial isolates and bacterial consortia

S.N.	Bacterial isolates	Absorbance of sample at 630 nm (As)	Absorbance of reference at 630nm (Ar)	% siderophore
1	SRP9 II 1	0.893	1.638	45.48
2	RRC I 5	0.773	1.638	52.80
3	MRC II 6	0.913	1.638	44.26
4	RRN II2	0.746	1.638	**54.45**
5	RRCII3	0.919	1.638	43.89
6	SRK14	0.960	1.638	41.39
7	Consortia 1	0.526	1.638	**67.88**
8	Consortia 2	0743	1.638	54.63
9	Consortia 3	0.840	1.638	48.71
10	Consortia 4	0.630	1.638	61.53
11	Consortia 5	0.674	1.638	58.85

4.7.3 Zinc solubilization

Zinc solubilizing bacteria solubilize complex zinc compounds by production of organic acids, sequest the zinc cations and decrease the pH **(Alexander, 1977).** All six isolates and consortia were further screened for zinc solubilization activity by formation of clear halo zone on basal agar medium supplemented with 0.1% $ZnCO_3$ and ZnO. All six bacterial isolate and five bacterial consortia were highly efficient and showed clear halo zone around bacterial colony after 4-5 days of incubation **(Table 4.8).** Individually MRCII 6 showed highest Zn solubilization efficiency i.e. 233.3 % in ZnO and 300 % in $ZnCO_3$ amended medium **(Table 4.8; Figure 4.4a and b).** Consortia 1 showed maximum efficiency of ZnO solubilization i.e. 300 %. Whereas in $ZnCO_3$ supplemented medium the efficiency was 260%. The results of the present study confirmed that microbial consortia solubilize more zinc as compared to single bacterial isolate.

Table 4.8: Zinc solubilization potential of bacterial isolates on plate assay

S.N.	Bacterial isolates	Zinc oxide (ZnO)			Zinc Carbonate ($ZnCO_3$)		
		Colony Diameter (cm)	Clearing Zone (cm)	Solubilization efficiency (%)	Colony Diameter (cm)	Clearing Zone (cm)	Solubilization efficiency (%)
1	SRP9II1	0.3	0.5	166.6	0.3	0.6	200
2	RRCII3	0.6	0.7	116.6	0.5	0.6	120
3	RRCI5	0.3	0.7	233.3	0.3	0.9	**300**
4	RRNII2	0.3	0.6	200	0.4	0.8	200
5	MRCII6	0.3	0.5	80	0.4	0.6	150
6	SRK14	0.3	0.3	100	0.3	0.4	133.3
7	Consortia 1	0.5	1.5	**300**	0.5	1.3	260
8	Consortia 2	0.5	1.3	133	0.7	0.5	71.4
9	Consortia 3	0.6	1.2	200	0.5	1.1	220
10	Consortia 4	0.5	1.2	240	0.5	0.8	160
11	Consortia 5	0.3	0.5	166.6	0.3	0.6	200

4.7.4 Quantification of zinc solubilization by AAS

All six bacterial isolates and bacterial consortia were further tested for their zinc solubilization potential in broth assay amended with ZnO and $ZnCO_3$. Zinc solubilization after 14 days of incubation all the consortia and bacterial isolates showed significantly better potential Zn solubilization and the values of solubilizing zinc was at par for both ZnO and ZnCO3 amended medium, except SRK14 which showed lowest Zn solubilization i.e. 10.51mgL^{-1} and 4.97 mgL^{-1} in ZnO and $ZnCO_3$ amended broth, respectively **(Table 4.9)**. In ZnCO3 amendment broth, consortia 2 showed highest Zn solubilization 17.32±0.01 mgL^{-1}. Present study also showed a different solubilization potential that were found among the different microbial isolates in ZnO and $ZnCO_3$ containing media. Zinc solubilization in broth and in plate assay was improved in consortia as compare to single bacterial isolates.

Table 4.9: Zinc solubilizing potential of zinc solubilizing bacterial (ZSB) isolates and consortia with different insoluble zinc compounds

S.N.	Treatments	ZnO (mgL^{-1})*	$ZnCO_3$ (mgL^{-1})*
1	**SRP9II1**	**18.31**	15.77
2	**RRCII3**	**18.31**	14.66
3	**RRCI5**	15.53	16.34
4	**RRNII2**	15.73	15.77
5	**MRCII6**	**18.30**	**17.26**
6	**SRK14**	10.51	4.97
7	**Consortia 1**	**18.31**	15.75
8	**Consortia 2**	16.76	**17.26**
9	**Consortia 3**	18.13	17.20
10	**Consortia 4**	18.24	**17.32**
11	**Consortia 5**	16.95	15.77
	C.D. (5%)	0.54	0.42
	SE(m)	0.18	0.14
	C.V. (%)	1.77	1.48

*Value indicates the mean of three replicates

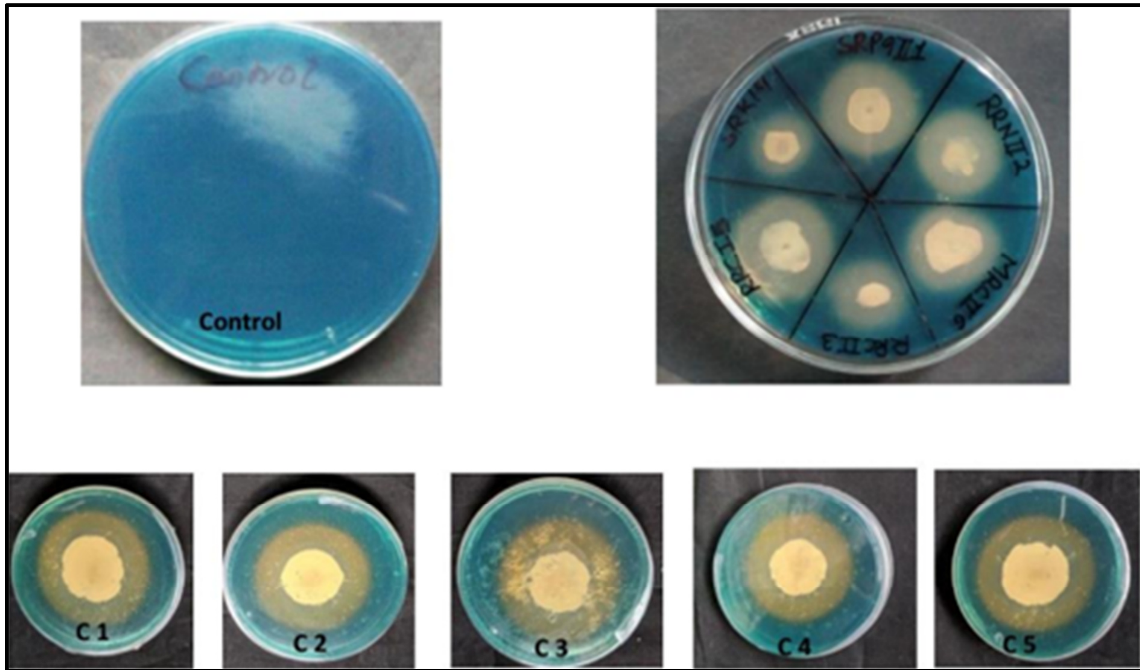


Fig 4.3: Siderophore production by consortia and bacterial isolates

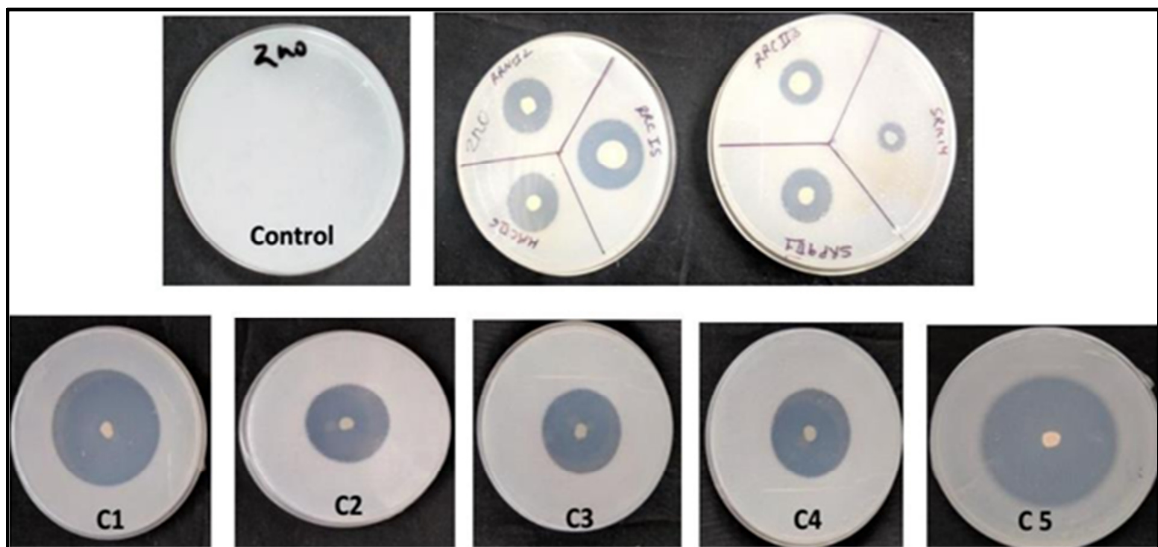


Fig 4.4 a): ZnO solubilization potential of consortia and bacterial isolates

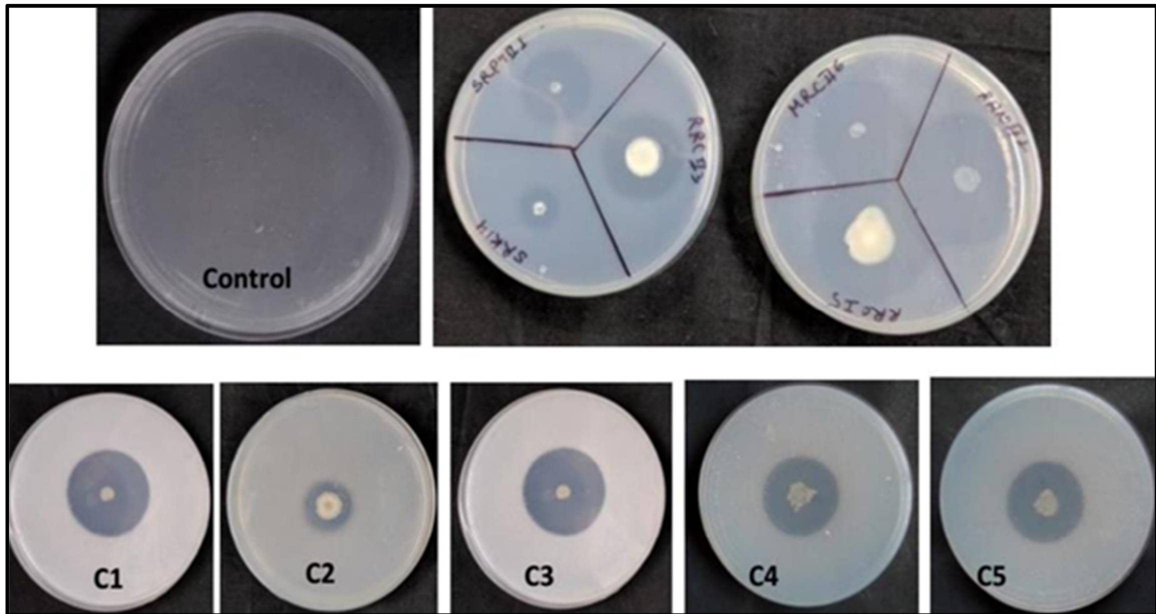


Fig 4.4 b): $ZnCO_3$ solubilization potential of consortia and bacterial isolates

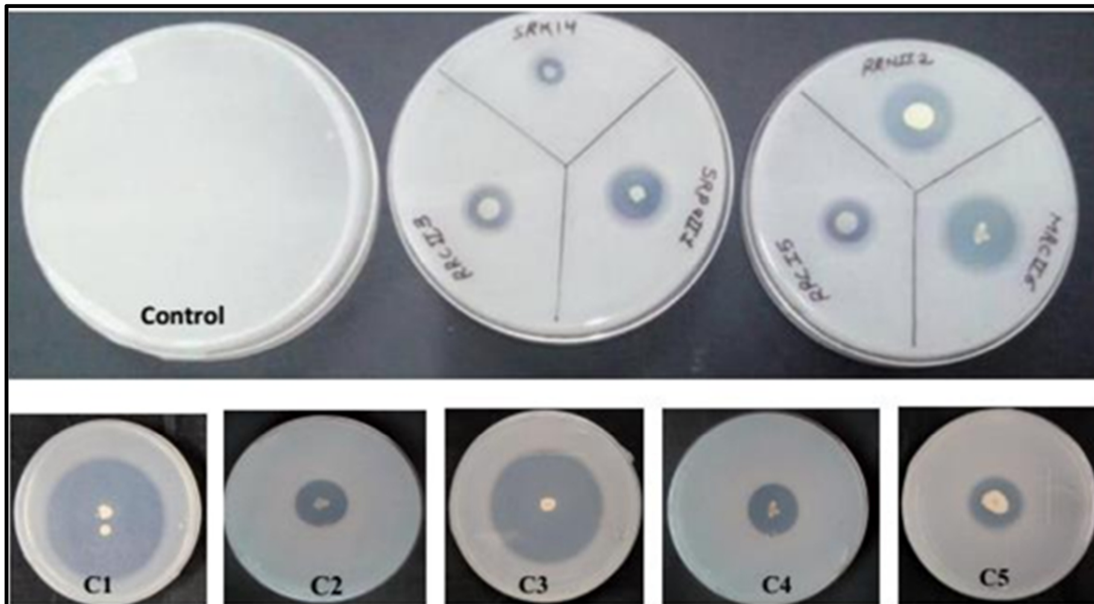


Fig 4.5 a): Phosphate solubilization by consortia and bacterial isolates

4.7.4. Phosphate solubilization

The phosphate solubilizing ability of all six test bacterial isolates and consortia were determined by formation of clear halo zone on pikovaskaya's agar medium supplemented with TCP (tri-calcium phosphate). All six test bacterial isolates showed the formation of clear halo zone out of which MRCII6 showed highest solubilization efficiency of 475% **(Table 4.10)**. However, Consortia 1 showed maximum phosphate solubilization followed by consortia 3 i.e. 233.3% and 216.6%, respectively **(Table 4.10; Figure 4.5 a)**.

Table 4.10: Phosphate solubilization potential of bacterial isolates

S.N.	Bacterial isolates	Phosphate solubilization		
		Colony diameter (cm)	Clearing Zone (cm)	Solubilization efficiency (%)
1	**SRP9 II 1**	0.6	1.8	**300**
2	**RRC I 5**	1.2	1.0	83.3
3	**MRC II 6**	0.4	1.9	**475**
4	**RRN II2**	0.6	0.6	100
5	**RRCII3**	0.4	0.5	80
6	**SRK14**	0.4	0.3	75
7	**Consortia 1**	0.6	1.4	**233.3**
8	**Consortia 2**	0.8	1.0	125
9	**Consortia 3**	0.6	1.3	216.6
10	**Consortia 4**	0.9	0.7	145.4
11	**Consortia 5**	0.6	0.8	133.3

4.7.3) Quantitative estimation of P solubilization

Phosphate solubilizing activity was estimated by using NBRIP broth supplemented with 1000 µg insoluble P/ml in the form of TCP at pH 6.8. NBRIP broth reported to have higher efficiency as compared to Pikovaskaya's medium (**Nautiyal,**

1999), has been used by various other workers (**Johri** ***et al.*,** **1999; Chatli** ***et al.*,** **2005; Singh** ***et al.*,** **2013**). After inoculation with P-solubilizing bacteria, the insoluble phosphate was solubilized and measured as soluble phosphate. The amount of P solubilization by six bacterial isolates and five consortia are presented in **Table 4.11; Figure 4.5b.**

Table 4.11: Quantitative assay of phosphate solubilization and pH changes exhibited by different bacterial isolates and their consortia in NBRIP broth medium

S.N.	Treatments	3th day of incubation		5th day of incubation		7th day of incubation		9th day of incubation	
		pH	Conc. (µg/ml)	pH	Conc. (µg/ml)	pH	Conc. (µg/ml)	pH	Conc. (µg/ml)
1	**SRP9 II 1**	4.7	149.9	4.0	166.5	2.5	343.6	3.3	288.8
2	**RRC I 5**	4.3	166.4	3.7	187.1	2.8	297.3	4.3	277.3
3	**MRC II 6**	4.6	142.5	5.3	142.8	3.8	170.9	3.5	204.9
4	**RRN II2**	4.5	174.0	3.7	203.8	3.3	202.3	4.2	182.0
5	**RRCII3**	6.1	112.6	4.7	166.8	4.8	151.2	5.8	137.8
6	**SRK14**	5.2	118.6	4.4	142.9	3.5	246.9	3.3	243.4
7	**Consortia 1**	3.7	201.6	3.4	245.4	2.4	385.0	3.1	332.4
8	**Consortia 2**	4.3	195.1	4.8	125.4	3.5	247.9	3.4	248.2
9	**Consortia 3**	5.5	156.7	3.6	198.3	2.5	302.6	3.2	282.8
10	**Consortia 4**	4.4	156.5	4.0	164.3	3.4	247.0	4.4	211.2
11	**Consortia 5**	4.1	177.0	2.6	381.8	4.4	175.5	3.3	164.7

Value indicates the mean of three replicates

Maximum isolates and consortia showed highest solubilization on the 5th and 7th day of incubation and their maximum values were varied from 166.8 to 385.0 µg P/ml, where maximum phosphate solubilization was shown by consortia 1 i.e. 385.0 µg P/ml at 7th day of incubation. Consortia 5 showed maximum phosphate solubilization i.e. 381.8 µg P/ml at 5th day of incubation. After a maximum point of solubilization bacteria and consortia showed decrease in solubilization efficiency. Several other workers also

reported such an increasing and decreasing trend in phosphate solubilization **(Gaur, 1990; Yadav and Singh, 1991; Goenadi *et al.,* 2000).**The reason for this trend may be attributed to the fact that when the rate of uptake of phosphate is higher than that of solubilization, a decrease in phosphate concentration in the medium could be observed. On the contrary, when the uptake rate decreases, the level of phosphate in the medium increases **(Rodriguez and Fraga, 1999).**The decrease in soluble phosphorus at later incubation period might be due to decreased solubilizing activity of microorganisms or increased P-absorption. Change in pH of the bacterial culture as well as consortia were observed with incubation days from initial value of 6.8 pH to 2 pH was observed to be lowest on the day of maximum of solubilization. **Vora and Shelat (1996)** also reported similar changes in the pH of the growth medium. A drop in pH of the broth during solubilization of inorganic phosphatic compounds has also been reported by various workers **(Gerretsen, 1948; Ahmad and Jha, 1968; Pandey *et al.,* 2006).**

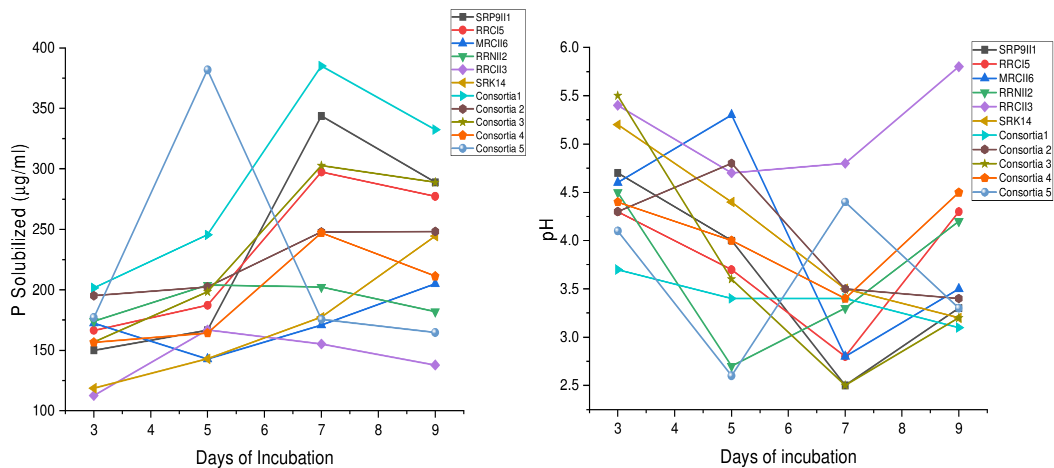


Fig 4.4 b: Trend of a) phosphate solubilizing kinetics with b) pH changes as exhibited by efficient isolates and bacterial consortia.

4.8. Functional characterization of drought tolerant bacterial isolates and bacterial consortia

All six drought tolerant isolates and bacterial consortia were further tested for their functional characteristics such as IAA production, HCN production and ammonia production **(Table 4.12).**

Table 4.12: Functional characteristics of bacterial isolates

S.N.	Treatments	Test for functional characteristics		
		HCN production	Ammonia production	IAA production
1	SRP9 II 1	-	+	+
2	RRC I 5	-	+	+
3	MRC II 6	-	+	+
4	RRN II 2	-	+	+
5	RRC II 3	-	+	+
6	SRK 14	-	+	+
7	Consortia 1	-	+	+
8	Consortia 2	-	+	+
9	Consortia 3	-	+	+
10	Consortia 4	-	+	+
11	Consortia 5	-	+	+

4.8.1. IAA production

In the present study IAA production by of single bacterial isolate and consortia was carried out. It was found that all selected bacterial culture and consortia were able to produce IAA **(Table 4.12.1; Figure 4.6 a)**. SRK14 showed highest production of IAA i.e. 165.4±1.13 µg/ml whereas in co-inoculation with MRCII6 the IAA production in decreased **(Table 4.12.1; Figure 4.6 a)**. Same as with RRRCI5 individually produces 41.82 µg/ml IAA whereas in consortia 2 and 5 co–inoculation with SRP9II1 and RRCII2 respectively the IAA production decreases (**Table 4.12.1; Figure 4.6 a**). Production of auxins (IAA) depends upon the strain and age of the microorganism. The promotion and expansion of root growth is one of the major markers by which the beneficial effect of plant growth promoting bacteria is measured **(Glick, 1995).** It has been reported by various workers that the precursor, L-tryptophan is necessary for IAA production by microorganisms (**Bent *et al.*, 2001; Asghar *et al.*, 2002; Park *et al.*, 2005; Tsavkelova *et al.*, 2007**). Supplementation of culture medium with tryptophan helps the microorganisms to produce IAA. In the present study also, the assessment of IAA was performed in the presence of L-tryptophan. Under natural conditions, L-tryptophan may be available in root exudates as noticed by **Beniziri *et al.* (1998)** which is inducing the microorganisms to produce IAA in the rhizosphere.

Table 4.12.1: Quantitative estimation of IAA production by bacterial isolates and consortia

S.N.	Bacterial isolates	IAA production (µg/ml)*
1	**SRP9 II 1**	22.5
2	**RRC I 5**	41.82
3	**MRC II 6**	14.19
4	**RRN II2**	15.52
5	**RRCII3**	21.74
6	**SRK14**	**165.4**
7	**Consortia 1**	27.92
8	**Consortia 2**	35.16
9	**Consortia 3**	33.57
10	**Consortia 4**	42.94
11	**Consortia 5**	39.57
	SE(m)	1.62
	C.D. (5%)	0.53
	C.V.	2.09

*Value indicates the mean of three replicates

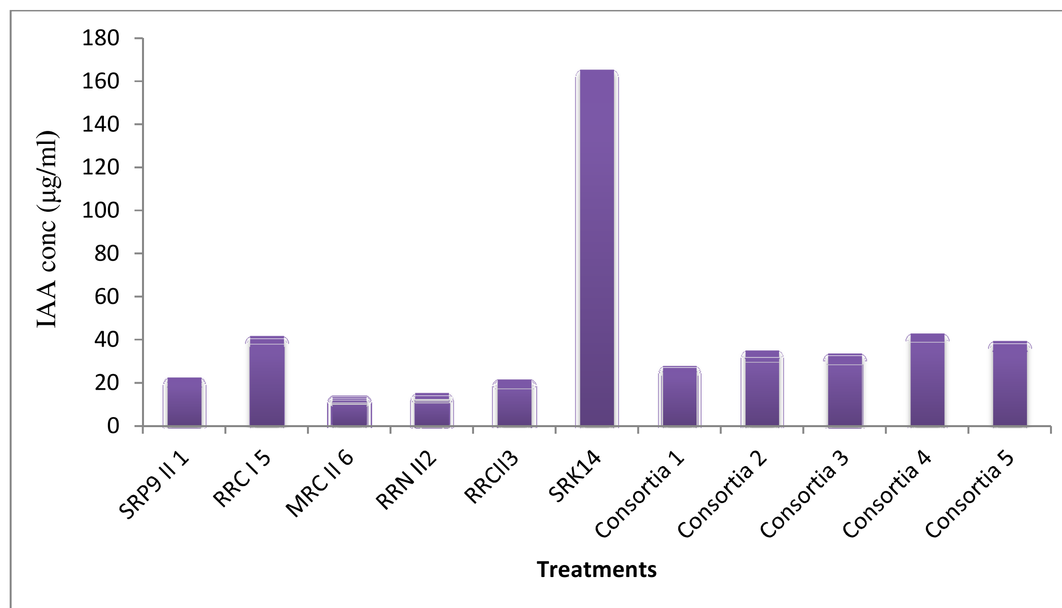


Fig- 4.5a): Quantitative estimation of IAA production by bacterial isolates and bacterial consortia

4.8.2. Ammonia production

Ammonia production is an important characteristic of PGPR, which indirectly influences plants growth **(Yadav *et al*., 2010).** Ammonia production assay was carried out for single bacterial isolate as well as in bacterial consortia. All six selected bacterial isolates and consortia were positive for ammonia production **(Table 4.12; Figure 4.6 b).** Ammonia also considered one of the plant growth promoting substances produced by various microbes inhabiting rhizosphere.

4.8.3. HCN production

Hydrogen cyanide is secondary metabolite, part of antifungal compounds produced by plant growth promoting rhizobacteria and involved in biological control **(Hass and Defago, 2005).** HCN affects cellular functionality by blocking cytochrome oxidase pathway and inhibit growth of the other microorganism **(Defago *et al*., 1990).** However, role of cyanide production is contradictory as it may be related with beneficial as well as deleterious rhizobacteria **(Bakker and Schippers, 1987; Alstrom and Burns, 1989).** In the present study no bacterial isolates and consortia were found to produce HCN.

4.9. Antibiosis assay

Antibiosis has been postulated to play an important role in disease suppression by rhizobacteria **(Gutterson *et al*., 1986).** In the present study, out of six bacterial isolates, four showed inhibition of mycelial growth of *Fusarium oxysporum* **(Table 4.1; Figure 4.7),** while no bacterial isolate were found to suppress mycelial growth of *Sclerotium rolfsi, Fusarium oxysporum* fungi causes head blight of wheat, on the other hand Southern blight, caused by the soilborne fungus *Sclerotium rolfsii.* Many rhizospheric bacterial strains have ability to inhibit mycelial growth of phytopathogens of crops via production of various extracellular enzymes, antibiotics, nutrition competitions, antibiosis parasitism and induced systemic resistance **(Whipps, 2001)** employing biocontrol activity and protect the crop plants from the adverse effect of the phytopathogens**.** Antimicrobial compounds produced by *Pseudomonas cepacia* were reported to inhibit the radial growth of some important soil borne pathogens like *Fusarium oxysporum, Macrophomina phaseolina, Sclerotium rolfsii, Rhizoctonia solani* and *Pythium ultimum* **(Baligh *et al*., 1999).**

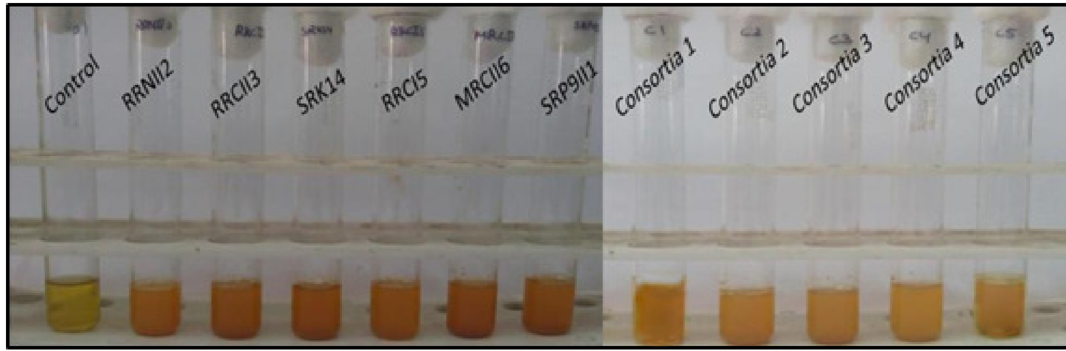


Fig 4.6 b): Ammonia production by consortia and bacterial isolates

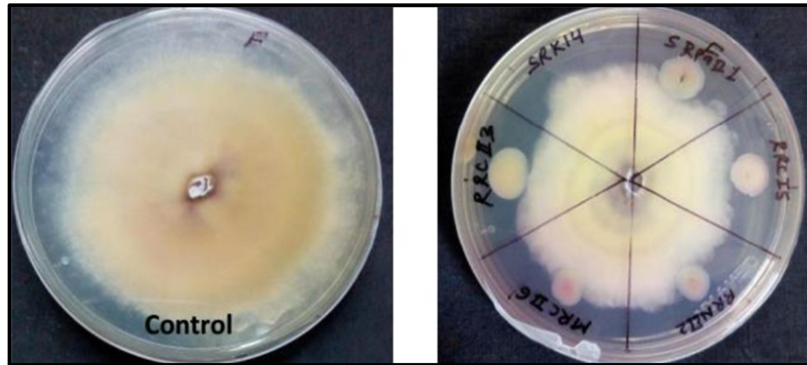


Fig 4.7: Antagonistic activity of bacterial isolates against *Fusarium oxysporum*

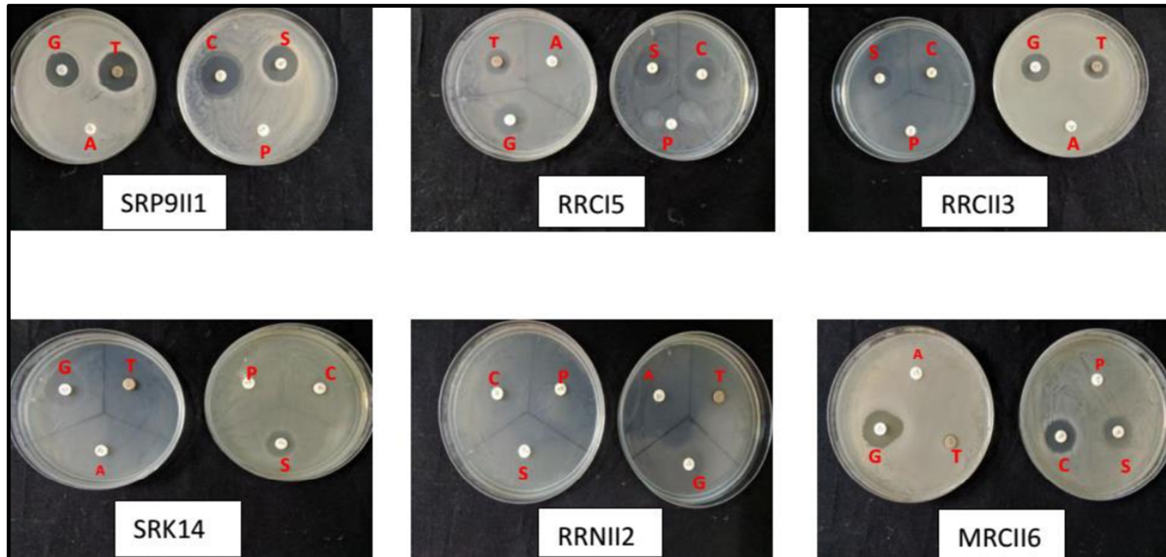


Fig 4.8: Antibiotic sensitivity test of bacterial isolates

T- Tetracycline (30µg/disc), **A**- Ampicillin (10µg/disc)

P- Penicillin (10µg/disc), **G**- Gentamicin (10µg/disc)

S- Streptomycin (25µg/disc), **C**- Chloramphenicol (50µg/disc)

Table 4.13: Antagonistic activities of bacterial isolates against plant pathogenic fungi

S.N.	Bacterial isolates	*Fusarium oxysporum*	*Sclerctonia rolfsii*
1	SRP9 II 1	+	-
2	RRC I 5	+	-
3	MRC II 6	-	-
4	RRN II2	+	-
5	RRCII3	+	-
6	SRK14	-	-

4.10. Antibiotic sensitivity test

In the present study antibiotic sensitivity test of bacterial isolates was checked by using antibiotics disc (Penicillin, Chloramphenicol, Gentamicin, Ampicillin, Streptomycin, Tetracycline) from Himedia, India. The results were interpreted on the basis of formation of clear zone around the antibiotic discs on bacterial lawn. Formation of clear zone shows sensitivity of bacteria towards particular antibiotics.

Table 4.14: Antibiotic sensitivity test of bacterial isolates

S.N	Bacterial isolates	Penicillin (10µg/disc)	Chloramphenicol (50µg/disc)	Gentamicin (10µg/disc)	Ampicillin (10µg/disc)	Streptomycin (25µg/disc)	Tetracycline (30µg/disc)
1	SRP9II1	-	+	+	-	+	+
2	RRCI5	-	+	+	-	+	+
3	MRCII6	-	+	+	-	+	-
4	RRNII2	-	+	+	-	-	-
5	RRCII3	-	-	+	-	+	+
6	SRK14	-	-	+	-	+	-

+ = Sensitive - = Resistant

All bacterial strains were sensitive towards gentamicin. Except RRNII2 all bacterial isolates were sensitive towards streptomycin. SRP9II1, RRCI5 and RRCII3 show sensitivity towards tetracycline. RRCII 3 and SRK14 did not show sensitivity towards chloramphenicol, but remaining isolates were sensitivity towards chloramphenicol. All cultures were resistance towards penicillin and ampicillin **(Table 4.14; Figure 4.8).**

4.11. Greenhouse pot experiment

All of five prepared potential bacterial consortia were further selected for green pot experiment on late variety of wheat (*Triticum aestivum* var. PBW373) on the basis of their *in-vitro* plant growth promoting traits. After 21 days of emergence, all treatments were subjected to drought stress by discontinuous watering to pots.

4.11.1. Biochemical analysis of leaf

Drought affect plant growth negatively results in decrease in relative water content, total chlorophyll content, photosynthesis rate and increased proline content with the increase in the intensity of drought stress on wheat cultivars reported by **Keyvan (2010)** and **Abdoli *et al.* (2013). Din *et al.* (2011)** also reported reduction in chlorophyll content under drought stress conditions. Drought and other abiotic stresses are responsible to cause oxidative damage in plants by formation of ROS pose serious threat to the cell functioning by damaging lipids and proteins. **Moran *et al.* (1994)** reported four times increase in lipid and protein peroxidation under drought stress as compared with normal conditions in pea plant. Therefore, to examine the effect of inoculation of PGPR consortia in wheat plants under drought stress conditions various biochemical studies like chlorophyll content, carotenoid content, proline content, % relative water content, lipid peroxidation and catalase activity were performed to observe the effect of drought stress in growth and physiology of plants. Sampling was done at 45th and 75thday from the sowing of seeds.

4.11.1.1 Chlorophyll content and carotenoid content

In present study Chlorophyll **(Table 4.15; Figure 4.9 a, b, c and d)** and carotenoid content were estimated at 45th and 75th day. Consortia 1 showed highest chlorophyll a i.e. 3.67 mg/g which was 102.76% improved over negative control, chlorophyll b i.e. 1.97 mg/g which was 258.1% improved over negative control and total chlorophyll was 5.43mg/g which was 129.11% higher as compare to negative control. All consortia treated treatments showed improve chlorophyll content under drought stress conditions. Carotenoid content was highest in consortia 4 i.e. 0.41 μg/g FW, 57.69% higher than negative control at 45th whereas at 75th consortia 3 showed 0.51μg/g FW of carotenoid content which was 96.15% higher than negative control. Present study

Table 4.15 : Chlorophyll and carotenoid content in leaf sample

Treatments	Chl a (mg/g)				Chl b (mg/g)			
	*45th day	%increase over negative control	*75th day	%increase over negative control	*45th day	%increase over negative control	*75th day	%increase over negative control
Positive control	3.96	-	3.98	-	2.11	-	1.87	-
Negative control	1.81	-	1.98	-	0.55	-	0.59	-
Consortia1	**3.67**	**102.76**	**3.61**	**82.32**	**1.97**	**258.1**	0.85	65.45
Consortia2	3.38	86.74	3.38	70.70	0.81	47.27	1.20	103.3
Consortia3	3.19	76.24	3.66	84.84	0.85	54.54	0.85	44.06
Consortia4	3.5	93.37	3.33	68.18	0.91	65.45	0.8	44.06
Consortia5	2.76	52.48	3.45	74.24	1.20	118.18	**1.97**	**233.9**
SEm±	0.09		0.08		0.08		0.13	
CD (5%)	0.24		0.24		0.27		0.38	
CV%	4.26		4.12		12.83		19.03	

*Value indicates the mean of three replicates

Table 4.15 : Contd…

Treatments	Total chl (mg/g)				Carotenoid (µg/g)			
	*45th day	%increase over negative control	*75th day	%increase over negative control	*45th day	%increase over negative control	*75th day	%increase over negative control
Positive control	6.32	-	5.86	-	0.45	-	0.53	-
Negative control	2.37	-	2.57	-	0.26	-	0.26	-
Consortia 1	**5.43**	**129.11**	4.45	73.15	0.39	50	0.39	50
Consortia 2	4.26	79.74	4.52	75.87	0.37	42.30	0.42	61.5
Consortia 3	3.79	59.91	4.52	75.87	0.34	30.76	**0.51**	**96.15**
Consortia 4	4.42	86.49	4.13	60.7	**0.41**	**57.69**	0.36	38.46
Consortia 5	3.71	56.54	**5.43**	**111.28**	0.32	23.07	0.47	80.76
SEm±	0.13		0.03		0.02		0.06	
CD (5%)	0.40		0.12		0.07		0.02	
CV%	5.35		1.52		11.41		2.58	

*Value indicates the mean of three replicates

agreed with the findings of **Megala and Paranthaman (2017**), who observed the different treatments, inoculation of PGPR T11-consortium (*Azospirillum lipoferum* SAz 9+ *Azotobacter chroococcum* Sat 1+ *Bacillus megatirium* 18+ *Pseudomonas fluorescens* SPf21) recorded the maximum plant chlorophyll. Inoculation PGPR consortia (*Azospirillum*+ *Pseudomonas*) showed highest value for carotenoid content in normal and stress conditions **(Ahmadi *et al*., 2013).**

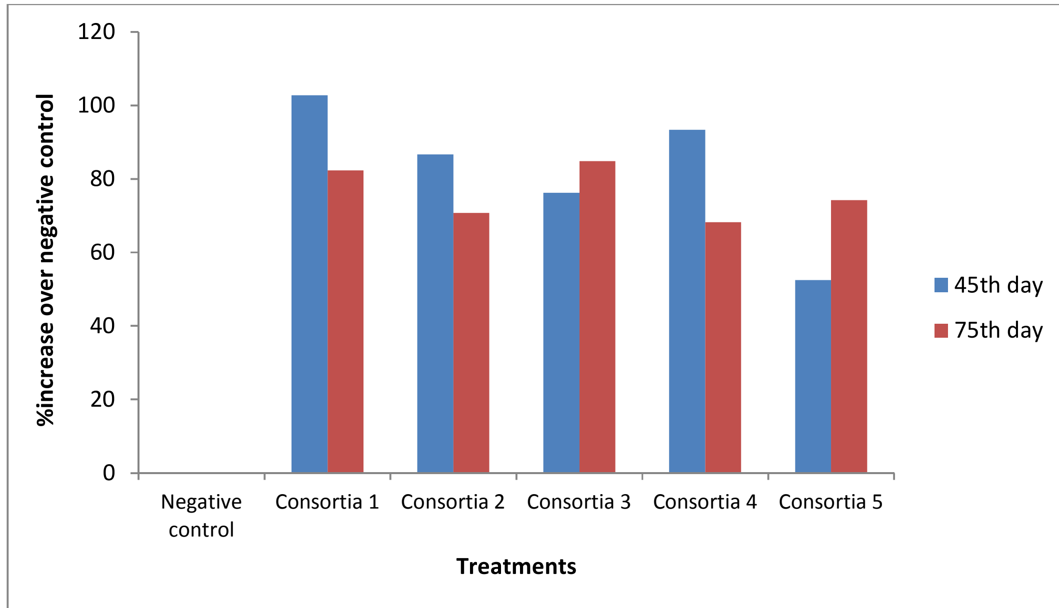


Fig 4.9 a) Chlorophyll a content (% increase over negative control) at 45th and 75th day

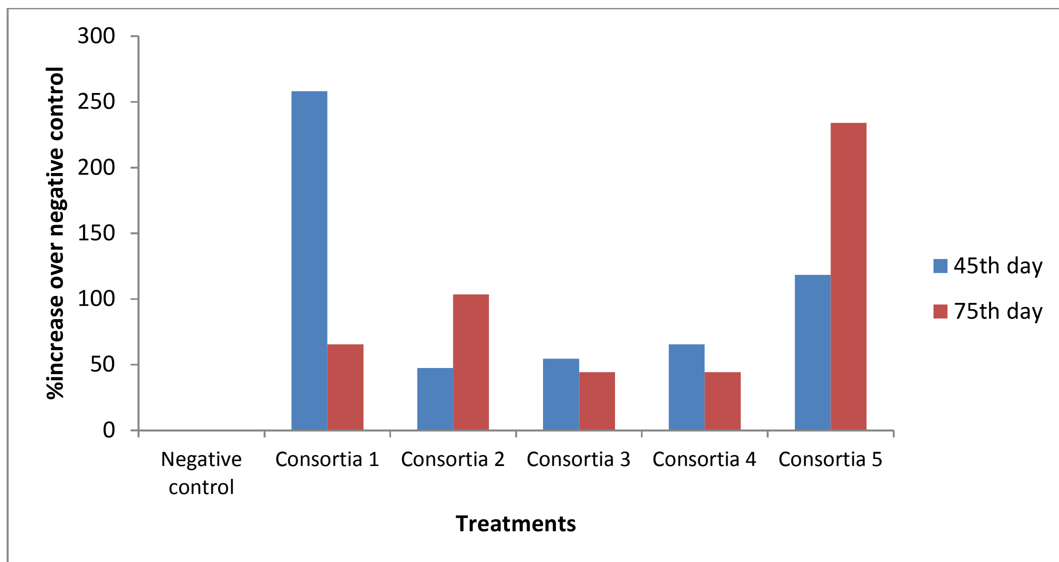


Fig 4.9 b) Chlorophyll b content (% increase over negative control) at 45th and 75th day

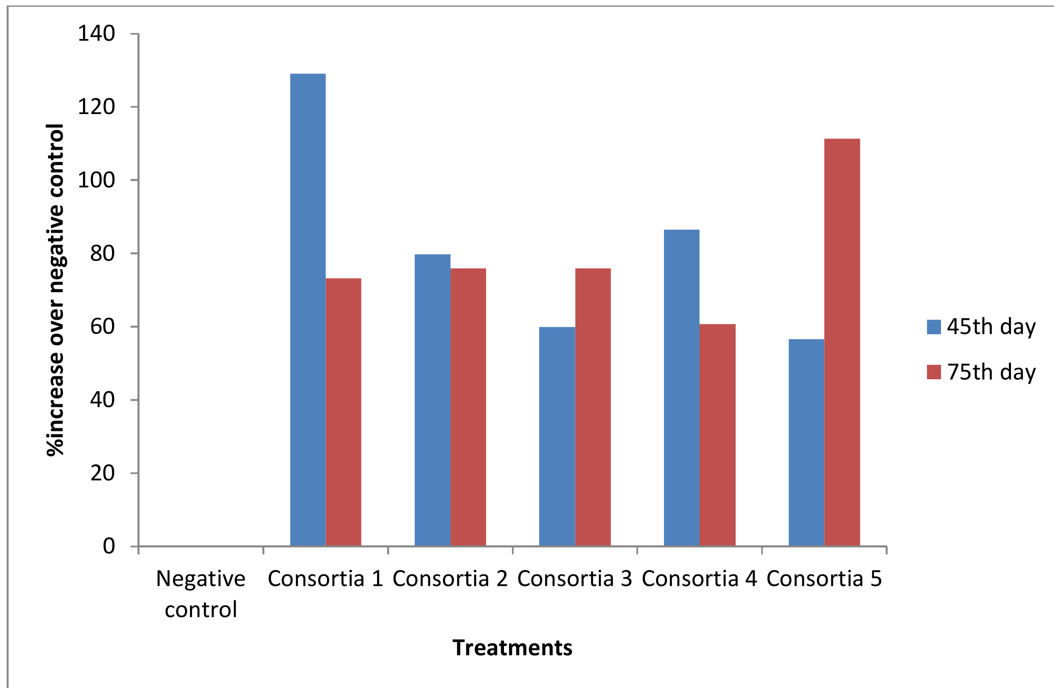


Fig 4.9 c) Total chlorophyll content (% increase over negative control) at 45th and 75th day

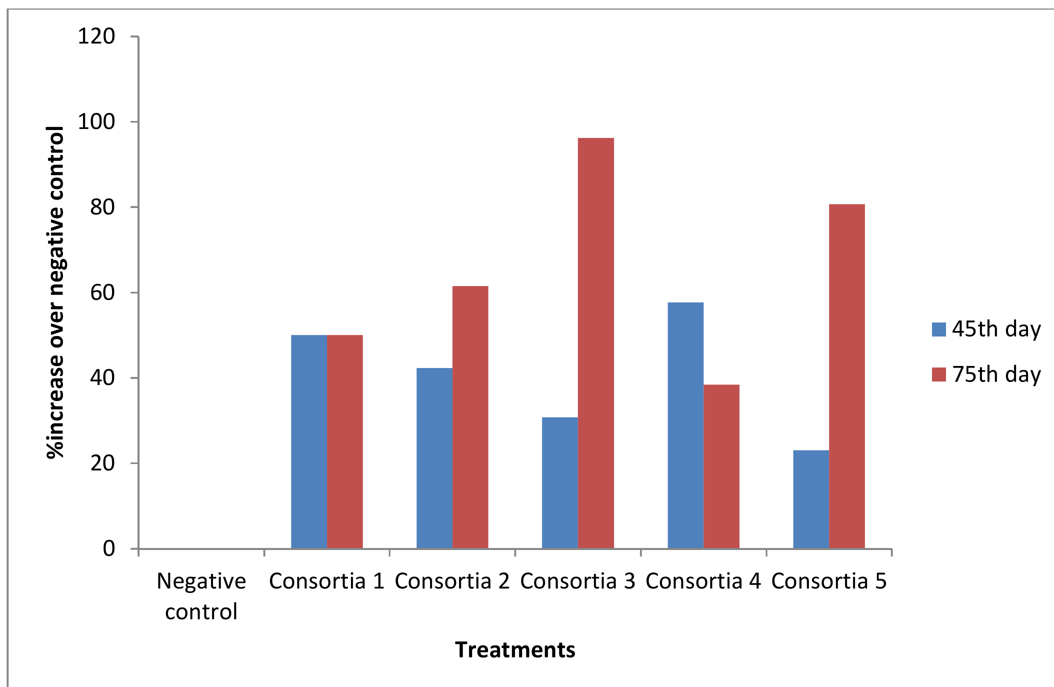


Fig 4.9 d) Carotenoid content (% increases over negative control) at 45th and 75th day

4.11.1.3. Relative water content

Relative water content was determined to check plant water status,**(Table 4.16; Figure 4.10)** shows the effect of different treatments on the % relative water content in leaf samples at 45 and 75 days interval. The plant treated with bacterial consortia 1 showed highest i.e. 78.45% and 80.56% which was enhanced by 43.4% and 41.35% over negative control at 45th and 75th day respectively. However all inoculated treatment also significantly showed significantly improved the relative water content as compared with negative control. **Cura *et al.* (2017)** shows the RWC (%) of the plants according to inoculation status and hydric conditions. The RWC was higher in plants inoculated with either PGPR in both watered and drought conditions. In the watered condition, plants inoculated with *Azospirillum brasilense* had the highest RWC (10% higher) versus control plants. In the drought condition, plants inoculated with *Herbaspirillum seropedicae* had the highest RWC (5.5% higher) versus control plants.

4.11.1.4. Catalase activity

In the present study, the experiment was carried out to determine the level of catalase activity in plants inoculated with bacterial consortia **(Table 4.16; Figure 4.11).** At 45th and 75th day Consortia 4 showed highest catalase activity i.e. 43.91 and 59.87, respectively which was increased by 85.04% at 45th day and 128.51% at 75th day, compared with negative control. With respect to the negative control all treatments showed improved catalase activity. Positive control showed lowest catalase activity indicates non stress conditions due to regular watering. Increased catalase activity in drought conditions in plants inoculated with PGPR consortia, might indicate that catalase is major enzyme detoxifying hydrogen peroxide in wheat leaves. Similar results were reported by **Heidari and Golpayegani (2012)** and **Sairam *et al.* (2005)** for other abiotic stresses.

Table 4.16: Relative water content and catalase activity in leaf sample

	Relative water				Catalase activity			
Treatments	***45th day**	**%increase over negative control**	***75th day**	**%increase over negative control**	***45th day**	**%increase over negative control**	***75th day**	**%increase over negative control**
Positive control	87.44	-	84.29	-	14.15	-	15.49	-
Negative control	54.70	-	56.99	-	23.73	-	26.20	-
Consortia 1	**78.44**	**43.4**	**80.56**	**41.35**	31.93	34.55	28.32	8.09
Consortia 2	76.12	39.15	80.33	40.95	30.28	27.60	37.36	42.59
Consortia 3	69.19	36.15	75.50	3247	41.81	76.19	36.58	39.61
Consortia 4	57.49	5.10	67.99	19.30	**43.91**	**85.04**	**59.87**	**128.51**
Consortia 5	61.62	12.65	70.33	23.40	39.91	68.18	35.12	34.04
SEm±	0.77		0.94		028		0.45	
CD (5%)	2.34		2.85		0.87		1.37	
CV%	1.93		2.20		0.54		2.29	

*Value indicates the mean of three replicates

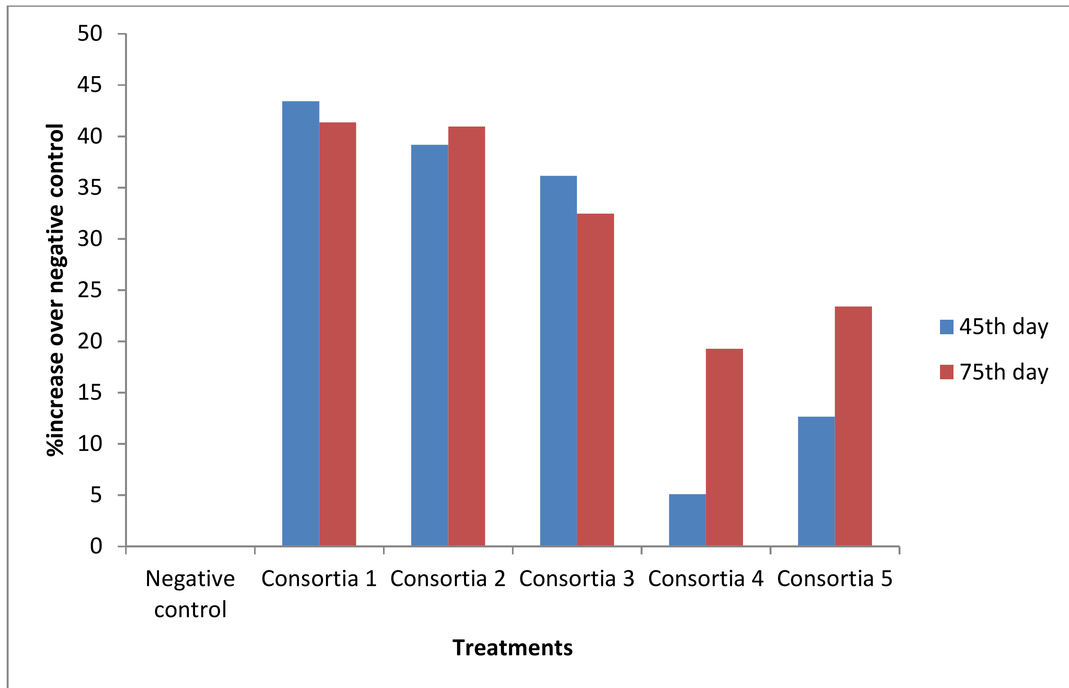


Fig 4.10: Relative water content (% increase over negative control) at 45th and 75th day

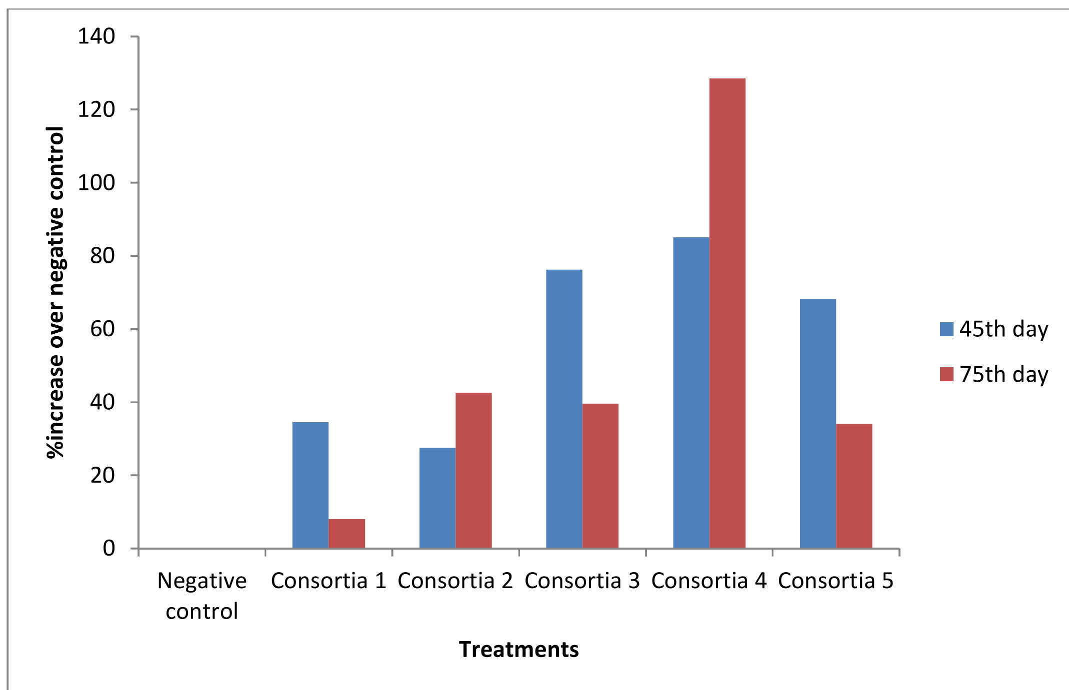


Fig 4.11: Catalase activity (% increase over negative control) at 45th and 75th day

4.11.1.5. Proline content

Proline is an indicator of osmoregulation, higher accumulation of proline in PGPR inoculated treatment indicates there higher tolerance to water stress conditions **(Gusain *et al.*, 2015)**. In present study proline content was measured during the experiment the results are indicated in **(Table 4.17; Figure 4.12)**. At 45th day treatment Consortia 5 showed 55.43% increase in proline accumulation i.e. 45.06 µmole/gm fw, as compared with the negative control and on 75th day Consortia 3 showed maximum proline accumulation 53.81±0.75 µmole/gm fw which was 64.35% higher compared with negative control. All five PGP bacterial consortia treated treatments in drought stress condition observed to accumulate more proline as compare to negative control. Similar results were reported by **Gusain *et al.* (2015)**, in severe drought stress conditions treatment inoculated with bacterial consortia showed higher proline accumulation. **Armada *et al.* (2014)** also reported that under drought stress conditions inoculation of PGPR *B. thuringiensis* (Bt) enhanced shoot proline content in *Lavandula dentate* compared to control plants under drought stress.

4.11.1.6. MDA content

MDA acts as a lipid peroxidation indicator in present study MDA was quantified in order to assess the integrity of the cellular membranes. It is known that in drought stress conditions membrane peroxidation in leaf tissues enhance, resulting in increase in the MDA content. Therefore, in the present study determination of the leaf MDA content was carried out in plants treated with potential bacterial consortia. MDA content **(Table 4.17; Figure 4.13)** in negative control was 18.23µmole g^{-1}FW and 16.13µmoleg^{-1}FWat 45th and 75th day, respectively which was highest among all the treatments. At 45th day consortia 5 showed 12.42µmoleg^{-1}FW MDA content which was 31.87% lower than the negative control, similarly, consortia 1 at 75th day showed 12.13µmole g^{-1}FW MDA content which was 24.79% lower than the negative control. All treatment inoculated with bacterial consortia showed less MDA content as compare to the negative control, indicating that inoculation with bacterial consortia helps in overcoming membrane damage. These results agree with **Wang *et al.* (2007),** who reported that MDA content in cucumber treated with BBS under drought stress conditions decreased by 38.5% compared with the control plants. MDA in the inoculated plants showed 15.9% decrease compared to the non-inoculated control treatments by **Chen *et al.* (2017)**.

Table 4.17: Proline content and MDA content in leaf sample

Proline content (µmole/g FW)					MDA content (µmole/g FW)			
Treatments	**45th day**	**%increase over negative control**	**75th day**	**%increase over negative control**	**45th day**	**%decrease over negative control**	**75th day**	**%decrease over negative control**
Positive control	23.82	-	20.86	-	7.88	-	6.23	-
Negative control	28.99	-	32.74	-	18.23	-	16.13	-
Consortia 1	43.21	49.22	50.97	55.68	14.55	-20.18	**12.13**	**-24.79**
Consortia 2	33.64	16.04	46.70	42.63	13.28	-27.15	13.20	-18.16
Consortia 3	40.11	38.35	**53.81**	**64.35**	16.37	-10.20	15.36	-4.77
Consortia 4	37.29	28.63	43.08	31.58	12.55	-31.15	16.00	-0.80
Consortia 5	**45.06**	**55.43**	50.36	53.81	**12.42**	**-31.87**	14.22	-11.844
SEm±	0.22		0.22		0.15		0.20	
CD (5%)	0.67		0.66		0.47		0.62	
CV%	1.06		0.89		2.00		2.68	

*Value indicates the mean of three replicates

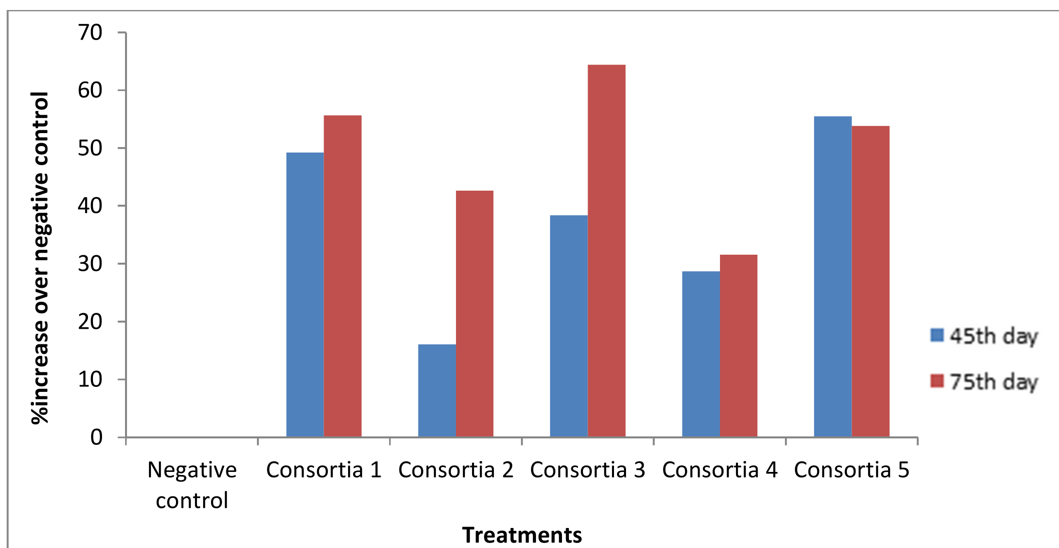


Fig 4.12: Proline content (% increase over negative control) at 45th and 75th day

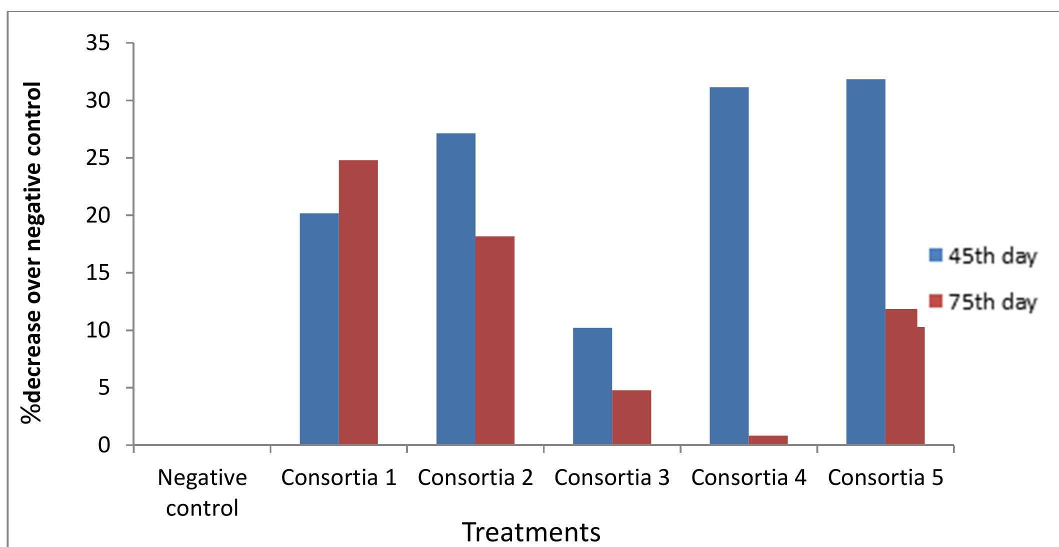


Fig 4.13: MDA content (% decrease over negative control) at 45th and 75th day

4.11.2. Effect of bacteria consortia on the growth characteristics of wheat.

Wheat seeds were treated with the bacterial consortia and the outcome of inoculation of potential plant growth promoting consortia on wheat expressed significant enhancement of plant growth parameters i.e. root length, shoot length, plant dry weight. Effect on wheat plant growth under inoculation of potential PGPR are shown in **Table 4.18; Figure 4.14.**

Table 4.18 Growth attributing characters of wheat plants

Treatments	*Shoot length (cm)	% increase over negative control	*Root length (cm)	% increase over negative control	*Plant dry weight (g)	% increase over negative control
Positive control	44.93	-	26.56	-	2.75	-
Negative control	37.26	-	18.23	-	1.85	-
Consortia 1	42.26	13.41922	24.33	33.46133	2.08	12.43243
Consortia 2	40.30	8.158884	22.76	24.84915	1.67	-9.72973
Consortia 3	44.26	18.7869	21.73	19.19912	1.92	3.783784
Consortia 4	44.66	19.86044	23.03	26.33022	2.56	38.37838
Consortia 5	40.50	8.695652	19.8	8.612178	1.88	1.621622
SEm±	0.68		0.75		0.18	
CD (5%)	2.08		2.27		0.57	
CV%	2.86		5.82		15.56	

*Value indicates the mean of three replicates

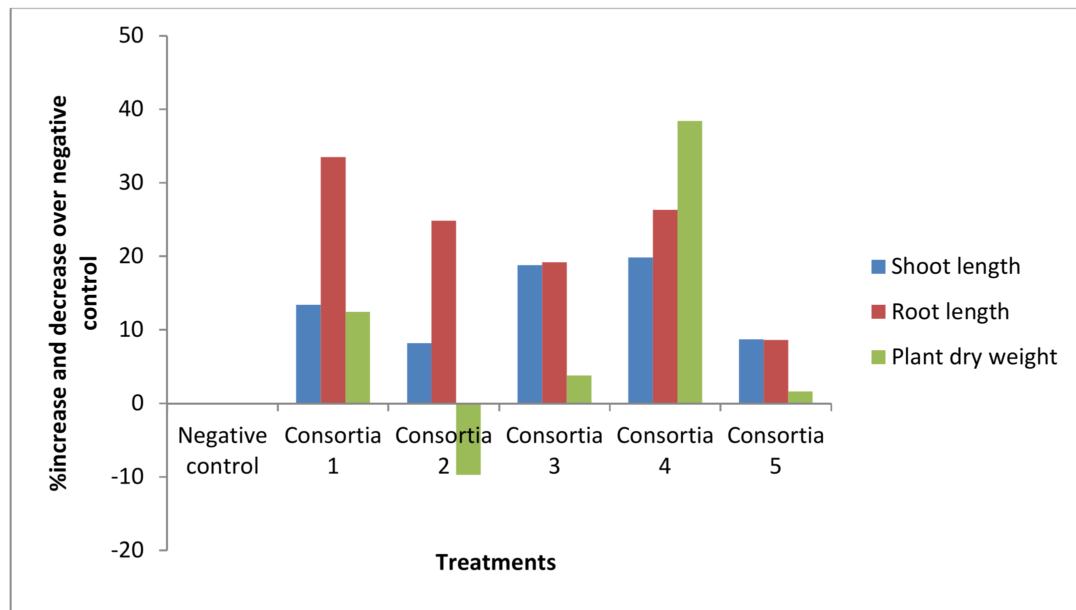


Fig 4.14: % increase and decrease in shoot length, root length and plant dry weight over negative control

To reduce the effect of drought stress and provide drought tolerance to plants PGPR significantly enhances the root length of plants. The effect of and Consortia 4 was maximum in respect to shoot length which was significantly increased by 19.86% over negative control even the effect of this consortium was at par compared to positive control (**Table 4.21; Figure 4.14**). However, the effect of the other consortium on shoot length was also significant compared to negative control and the least value was recorded for consortia 5, able to increase shoot length by 1.87% over negative control **(Table 4.18; Figure 4.14)** Maximum root length was observed in Consortia 1, which was significantly increased by 33.46% over negative control and decreased by 8.77% over positive control. Plant dry weight was highest for Consortia 4 was significantly increased by 38.37% over negative control and decreased by 6.9% over positive control **(Table 4.18; Figure 4.14)**. **Chen *et al.* (2017)** also reported similar results in which the plants inoculated with LTYR-11ZT showed 17.1%, 41.8% and 112% increase in shoot length, root length and total plant fresh weight, respectively, as compared to the non-inoculated stressed control. **Rajsekar and Elango (2011)** observed significant increased plant height and root length of *Withania somnifera* inoculated with consortia of *Bacillus*, *Pseudomonas*, *Azotobactor* and *Azospirillum,* compared to single inoculation and negative control.

4.12. Shelf-life of bacterial consortia in talc and sugarcane bagasse, based formulations

The bio-formulations generally composed of active material which must be preserved or maintained in viable condition to produce its biological effect these active material is mixed with carrier materials such as talc, clay, oil and water or others to develop the formulation. Moreover, the bio-formulations are safer to handle, easier to apply, storage and when required, can be applied at the site of action. Formulations are incorporated with, enrichment materials comprising of nutrient-rich medium such as, sucrose, trehalose, maltose and molasses. Formulations that transfer the growth promoting activities of rhizobacteria from laboratory to field have a major impact in agriculture. In the present study, the viability of consortium was evaluated in the form of talc and sugarcane bagasse based formulation for a period of 90 days stored at 4°C, room temperature and outside conditions under shade. Viability and population density of these formulations were tested in interval of 15 days, up to 90 days. Number of colonies appeared on NA for each dilution was counted. Results are recorded in **Table 4.19 a** and **b.**

In the present study results showed that maximum CFU of bio formulation of bacterial consortia was found in zero days as compare to other days and was seen up to 90 days. The initial population density of all talc and bagasse based formulation was 10^{10}/g. Gradual declining trend of population density was observed up to 90 days in all prepared formulation. Present study agreed with **Kumar *et al.* (2013)** who reported the same trend of gradually declining of cfu count with the time of storage in the different bioformulation.

In talc based bioformulation TL3 showed maximum cfu at 90th day i.e.159×10^{10} and TL1 showed lowest cfu i.e.178×10^{9}stored at 4°C. At room temperature TR5 and TR3 showed highest and lowest cfu i.e. 244×10^{8} and 88×10^{8} respectively. Talc based bioformulation stored outside under shade conditions showed maximum decrease in the cfu at 90th day where lowest cfu was observed in TO3 i.e.139×10^{7}. Moreover, in bagasse based formulation stored at 4°C BL1 showed highest cfu i.e. 261×10^{8} and lowest cfu was observed in BL5 105×10^{8}. At room temperature BR1 showed highest cfu i.e.$144\times10^{7}$and lowest cfu in BR3 i.e.170×10^{6}. Whereas, BO1 showed highest 84×10^{5} cfu and BO1 showed lowest 264×10^{5}cfu. Compare to sugarcane bagasse based formulation talc based formulation showed high efficiency to maintain viability of consortia stored in same conditions. Earlier workers reported shelf life of different formulation up to 9 and 6 months at different storage conditions. **Karunanithi *et al.* (2001)** reported shelf life of Talc and gypsum based formulation up to 150 days at room temperature.

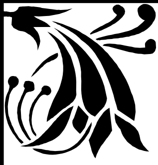
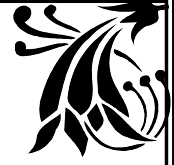
The shelf-life of Talc based formulation and sugarcane bagasse based formulations was relatively prolonged in cold storage (4°C) than at room temperature and outside conditions. At temperature below 5°C, the metabolic activities of microorganisms are ceased and the cell division is almost stopped **(Madigan and Martinko, 2006).** Storage of bio formulations in a storehouse without refrigerator in the range of 5 to 30°C usually causes reduction in microbial shelf life. **Sandikar and Awasthi (2010)** prepared Pseudomonas and Bacillus bioformulations and stored at room temperature (30°C) and cold conditions (4°C), after each month tested for viability and concluded that storage of bioformulations at 4°C was found significant than at room temperature.

Table 4.19 a): Shelf life of talc based formulation stored at different condition

Conditions	Formulation	CFU/g (days)						
		0	15	30	45	60	75	90
4°C	TL1	288×10^{10}	272×10^{10}	242×10^{10}	221×10^{10}	198×10^{10}	163×10^{10}	**178×10^{9}**
	TL2	272×10^{10}	256×10^{10}	175×10^{10}	169×10^{10}	265×10^{9}	219×10^{9}	193×10^{9}
	TL3	298×10^{10}	252×10^{10}	200×10^{10}	175×10^{10}	187×10^{10}	166×10^{10}	**159×10^{10}**
	TL4	252×10^{10}	236×10^{10}	201×10^{10}	184×10^{10}	148×10^{10}	255×10^{9}	180×10^{9}
	TL5	249×10^{10}	214×10^{10}	195×10^{10}	156×10^{10}	134×10^{10}	278×10^{9}	245×10^{9}
Room temperature	TR1	286×10^{10}	274×10^{10}	204×10^{10}	107×10^{10}	226×10^{9}	192×10^{9}	176×10^{8}
	TR2	298×10^{10}	253×10^{10}	202×10^{10}	249×10^{9}	208×10^{9}	179×10^{9}	156×10^{8}
	TR3	242×10^{10}	$252\times \times 10^{9}$	221×10^{9}	178×10^{9}	174×10^{8}	156×10^{8}	**88×10^{8}**
	TR4	242×10^{10}	244×10^{10}	236×10^{10}	170×10^{9}	136×10^{9}	105×10^{9}	134×10^{8}
	TR5	276×10^{10}	248×10^{10}	224×10^{10}	178×10^{10}	238×10^{9}	162×10^{9}	**244×10^{8}**
Outside in shade	TO1	289×10^{10}	222×10^{10}	142×10^{10}	228×10^{9}	176×10^{9}	226×10^{8}	180×10^{7}
	TO2	294×10^{10}	248×10^{10}	178×10^{10}	202×10^{9}	162×10^{9}	234×10^{8}	**261×10^{7}**
	TO3	284×10^{10}	214×10^{10}	118×10^{10}	186×10^{9}	159×10^{9}	108×10^{8}	**139×10^{7}**
	TO4	262×10^{10}	265×10^{10}	212×10^{10}	184×10^{10}	206×10^{9}	199×10^{8}	145×10^{7}
	TO5	272×10^{10}	238×10^{10}	200×10^{10}	164×10^{10}	217×10^{9}	184×10^{9}	228×10^{8}

Table 4.19 b): Shelf life of sugarcane bagasse based formulation stored at different conditions

Conditions	Formulation	CFU/g (days)						
		0	15	30	45	60	75	90
4°C	BL1	256×10^{10}	178×10^{10}	128×10^{10}	232×10^{9}	204×10^{9}	192×10^{8}	**244×10^{8}**
	BL2	297×10^{10}	234×10^{10}	212×10^{9}	162×10^{9}	94×10^{9}	186×10^{8}	148×10^{8}
	BL3	285×10^{10}	232×10^{10}	218×10^{10}	188×10^{10}	246×10^{9}	226×10^{9}	170×10^{8}
	BL4	239×10^{10}	241×10^{10}	174×10^{10}	218×10^{9}	184×10^{9}	208×10^{8}	176×10^{8}
	BL5	278×10^{10}	276×10^{10}	200×10^{9}	118×10^{9}	276×10^{8}	200×10^{8}	**105×10^{8}**
Room temperature	BR1	256×10^{10}	178×10^{10}	228×10^{9}	232×10^{8}	204×10^{8}	252×10^{7}	**144×10^{7}**
	BR2	297×10^{10}	234×10^{10}	212×10^{9}	162×10^{9}	94×10^{8}	186×10^{7}	128×10^{7}
	BR3	285×10^{10}	232×10^{10}	218×10^{10}	188×10^{9}	146×10^{8}	226×10^{7}	170×10^{6}
	BR4	239×10^{10}	241×10^{10}	174×10^{10}	218×10^{9}	184×10^{9}	218×10^{8}	176×10^{6}
	BR5	280×10^{10}	126×10^{10}	165×10^{9}	118×10^{8}	78×10^{8}	100×10^{7}	**184×10^{6}**
Outside in shade	BO1	239×10^{10}	210×10^{8}	182×10^{8}	204×10^{8}	217×10^{8}	117×10^{7}	**84×10^{5}**
	BO2	245×10^{10}	187×10^{9}	264×10^{8}	155×10^{8}	102×10^{7}	174×10^{6}	186×10^{5}
	BO3	267×10^{10}	144×10^{10}	168×10^{9}	134×10^{8}	148×10^{7}	194×10^{6}	122×10^{5}
	BO4	243×10^{10}	108×10^{9}	224×10^{8}	268×10^{7}	190×10^{7}	238×10^{6}	**264×10^{5}**
	BO5	234×10^{10}	192×10^{9}	186×10^{8}	264×10^{7}	126×10^{7}	141×10^{6}	86×10^{5}

Summary and Conclusions

SUMMARY AND CONCLUSION

Plant growth promoting bacteria, reside in the vicinity of root known as rhizobacteria. They adapt to tolerate drought stress and also help to plants to mitigate the impact of environmental stress or drought stress by means of various mechanisms to maintain the plant growth and productivity under water deficit conditions. Based on the area of affected land and number of people affected then among any other environmental stresses, drought is considered as most deleterious stress. Numerous physiological and morphological changes appears due to the effect of drought stress which includes enhanced osmotic potential, loss of stability and integrity of membrane, production of free radicals which reacts with macromolecules and leads to the dysfunction of such molecules. PGPR assist host plants to cope up drought stress and significantly help plant to promote vigor index, seedling emergence and productivity by the production of exopolysaccharide, ACC deaminase activity, osmolyte accumulation, phytohormone secretion, ion homeostasis, antioxidant enzyme production and induced systemic resistance. PGPR enhances the soil fertility and health by mobilization of macro and micro nutrients such as iron, zinc, phosphate, nitrogen etc by means of organic acid production. The present study was aimed to explore the determination of *in-situ* drought tolerant and plant growth promoting properties of five potential bacterial consortia and determining the shelf life of these consortia in two different carrier, stored in three different conditions. To carry out present study, initially eighteen cultures were obtained from departmental culture collection and all cultures were retrieved and screened for the drought tolerance ability. Out of which nine drought tolerant bacterial cultures were selected. Then these drought tolerant bacterial isolates tested for plant growth promoting characteristics such as siderophore production, zinc solubilization, phosphate solubilization, EPS production and ACC deaminase activity and finally six potential bacterial isolates were selected due to their marvelous properties. These selected six bacterial isolates than utilized for consortia development, on the basis of their bio-compatibility test five bacterial consortia were developed. Plant growth properties of consortia were determined over single bacterial isolates. Moreover, functional properties such as indole acetic acid, ammonia, HCN production

and antagonistic capacity of consortia were determined at par with bacterial isolates. To determine the beneficial effect, the consortia were subjected to *in situ* pot experiment under greenhouse conditions on wheat (*Triticum aestivum* var. PBW-373). These five consortia were further used for talc and sugarcane bagasse bio formulation preparation stored in different conditions for shelf life determination. Some salient findings of current study are summarized below:

- All retrieved bacterial isolates were screened, on the basis of appearance of growth on nutrient broth amended with ploy ethylene glycol (PEG).

- Screened nine drought tolerant bacterial isolates were further tested for plant growth promoting traits. Among nine bacterial isolates, six were positive for siderophore production, seven were positive for zinc and phosphate solubilization, five isolates were positive for ACC deaminase activity and all nine bacterial isolates positive for EPS production.

- Final six potential bacteria *viz*. SRP II 9 I, SRK14, MRC II 6, RRN II 2, RRC I 5 and RRCII3 were selected on the basis of their plant growth promoting traits 3 and utilized to develop five bacterial consortia on the basis of biocompatibility of strains with each other.

- Morphological characterization of screened six bacterial isolates conformed that all bacteria are gram negative, showed different colony morphologies and pigmentation on nutrient agar plate. Endospore staining of the bacterial isolates confirmed that no isolate was endospore producer.

- All six bacterial isolates were further characterized for their ability to produce extracellular enzymes, confirmed that only one isolate SRP9II1 was positive for amylase production. SRP9II1, RRCI5, MRCII6, RRNII2 were positive for gelatinase. However, all isolates were positive for catalase production. No bacterial isolates were positive for pectinase and laccase enzyme activity. RRCI5, RRNII2 and SRK14 were positive for cellulase. Only SRK14 was positive for H_2S production. MRCII6, SRP9II1, RRNII2 and SRK14 were positive for urease production. SRP9II1, MRCII6 and RRNII2 were positive for ceasinase, whereas SRP9II1, MRCII6, RRNII2 and SRK14 positive for lipase production.

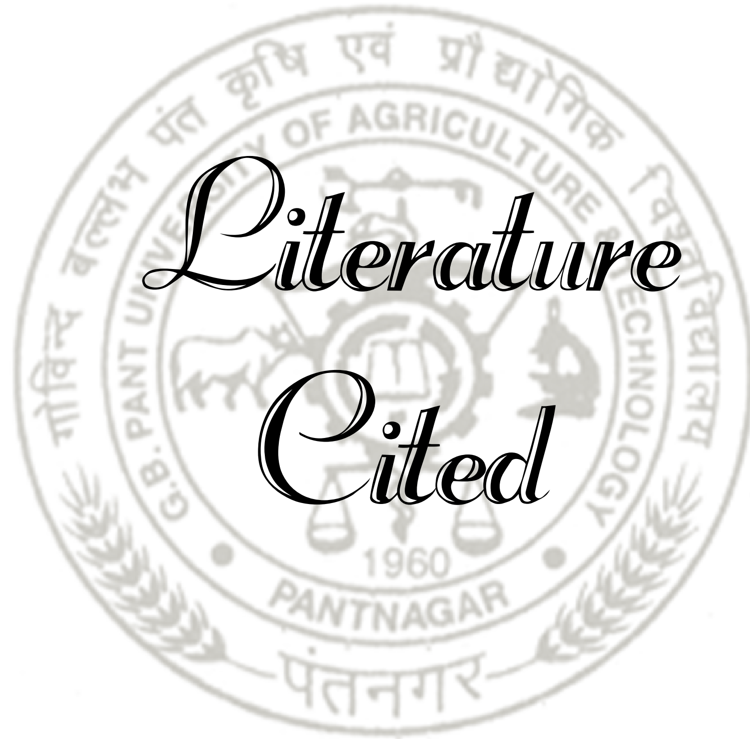
- In IMVIC test out of six bacterial isolates, only one bacterial isolates was positive for indole test, four were positive for methyl red test, three bacterial isolates were positive for voge's proskauer test, five were positive for citrate utilization. All bacterial isolates were also tested to metabolize various sugars. All the bacterial isolates were able to metabolize wide range of sugars via production of various enzymes, except SRK14 which only utilize glucose.
- Antibiosis assay was carried out by dual plate method for bacterial isolates and consortia, out of six bacterial isolates, four bacterial isolate showed inhibition of mycelial growth of *Fusarium oxysporum*, while no bacterial isolate were found to suppress mycelial growth of *Sclerotium rolfsii.*
- Antibiotic sensitivity was determined by disc diffusion method, using antibiotic discs [Tetracycline (3oµg/disc) ampicillin (10µg/disc), Gentamicin (10µg/disc), Chloramphenicol (50µg/disc), Streptomycin (25µg /disc), Penicillin-G (10µg/disc)]. No bacterial strains showed resistant towards gentamicin. Except RRNII2 all bacterial isolates were sensitive towards Streptomycin. All bacterial isolates were sensitive for gentamicin. Except RRCII3 and SRK14 all isolates showed sensitivity towards Chloramphenicol. All cultures were resistance towards Penicillin and Ampicillin.
- Comparative study between single bacterial isolates and consortia were carried out for siderophore production, zinc solubilization, phosphate solubilization, IAA production, ammonia production and hydrogen cyanide production, quantitatively and qualitatively
- Qualitative siderophore production efficiency of single bacterial and consortia were in the same range, Out of which RRCI5 showed highest efficiency i.e. 188.8% siderophores production. Siderophore production also quantified by liquid assay in which consortia 1 showed highest 67.88% siderophore unit whereas individual culture, RRNII2 showed maximum 54.45% siderophore unit.
- Zinc solubilization was tested on basal medium amended with 1% ZnO and ZnCO3. Individually MRCII6 showed highest Zn solubilization efficiency i.e. 233.3% in ZnO and 300% in $ZnCO_3$medium. Consortia 1 showed maximum efficiency i.e. 300% in ZnO and 260% in $ZnCO_3$medium. In broth assay of quantitative zinc solubilization after 14 day of incubation consortia 1, RRCII3,

MRCII6, SRP9II1 showed significantly highest Zn solubilization i.e. 18.31mgL^{-1} which was at par to each other, whereas, lowest Zn solubilization was observed in SRK14 i.e. 10.51 mgL^{-1} in ZnO and 4.97 mgL^{-1} in $ZnCO_3$ amendment broth.

- MRCII6 showed highest phosphate solubilization efficiency of 475%. Consortia 1 showed maximum phosphate solubilization followed by consortia 3 i.e. 233.3% and 216.6% respectively. However, in quantitative P solubilization most of the isolates and consortia showed highest solubilization on the 5th and 7^{th}day of incubation. Consortia 1 showed maximum phosphate solubilization i.e. 385 µg P/ml with pH 2 on 7^{th}day of incubation.
- All bacterial isolates and consortia were further tested for functional characterization such as ammonia production, IAA production, and HCN production. Exceptionally SRK14 showed highest production of IAA i.e. 165.4 µg/ml whereas in co-inoculation with MRCII6 in consortia 1 the IAA production in decreased to 27.92 µg/ml. same as with RRRC I5 individually it produces 41.82µg/ml IAA whereas in consortia 2 and consortia 5 co–inoculation with SRP9II1 and RRNII2, IAA production decreases. All bacterial strains and all five consortia were positive for ammonia production. Moreover, no bacterial isolates and consortia were found to produce HCN.
- Inoculation of all consortia of potential drought tolerant bacteria significantly improved plant growth in compared with negative control. Consortia 4 showed maximum shoot length which was significantly increased by 19.86% over negative control. Maximum root length was observed in consortia 1, which was significantly increased by 33.46% over negative control. Plant dry weight was highest for consortia 4 and increased by 38.37% over negative control.
- Talc and sugarcane bagasse based bioformulation of all five consortia were prepared and shelf life was determined up to 90 days stored at 4°C, room temperature and outside in shade. Talc based bioformulation maintain prolonged shelf life of bacterial consortia in all three conditions as compared with sugarcane bagasse based bioformulation.

In present era, climatic conditions are continuously changing due to natural and manmade activities. It leads to the various detrimental environmental stress such as drought stress, limits plant growth and productivity, plant associated drought

tolerant plant growth promoting rhizobacteria adapted various mechanisms to mitigate the effect of drought stress and to stimulate plant growth and productivity. In present study, consortia of potential drought tolerant bacteria showed significant plant growth promoting activity *in vitro* over single bacterial isolates. All the consortia significantly enhanced root and shoot length of wheat under drought stress conditions. Hence the present study may conclude that all consortia were able to confer drought tolerance in wheat plants under water deficit conditions. Among all consortia 1 and 4 showed marvelous plant growth promotion. Talc based bioformulation maintain higher shelf life, thus can develop as bioinoculant for drought susceptible crops to mitigate the effect of drought stress and to improve plant growth for sustainable agriculture practices.



Literature Cited

LITERATURE CITED

Abdoli, M., Saeidi, M., Jalali-Honarmand, S., Mansourifar, S. and Eghbal-Ghobadi, M. E. 2013. Evaluation of some physiological and biochemical traits and their relationships with yield and its components in some improved wheat cultivars under post-anthesis water deficit. *Environmental Stresses in Crop Sciences,* 61: 47-63

Ahemad, M. and Khan, M. S. 2012. Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard *Brassica compestris* rhizosphere. *Chemosphere*, 869: 945-950

Ahmad, F., Ahmad, I. and Khan, M. S. 2008. Screening of free-living rhizosphere bacteria for their multiple plant growth promoting activities. *Microbiological Research*, 163:173-181

Ahmad, M., Nadeem, S. M., Naveed, M. and Zahir, Z. A. 2016. Potassium-solubilizing bacteria and their application in agriculture. In: Meena, V.S., Maurya, B.R., Verma, J.P. and Meena, R.S. eds Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, 293-313

Ahmad, N. and Jha, K. K. 1968. Solubilization of rock phosphate by micro-organisms isolated from Bihar soils. *The Journal of General and Applied Microbiology*, 14: 89-95

Ahmed, E. and Holmstrom, S. J. M. 2014. Siderophores in environmental research:roles and applications.*Microbial Biotechnology*, 7: 196–208

Akinci, S. and Losel, D. M. 2012. Plant Water-Stress Response Mechanisms. In: Rahman, IMM, Hasegawa, H. eds. *Water Stress*, Croatia: intechweb.org, 15-42

Akiyoshi, D. E., Regier, D. A. and Gordon, M. P. 1987. Cytokinin production by *Agrobacterium* and *Pseudomonas* spp. *Journal of Bacteriology*, 1699: 4242- 4248

Alami, Y., Champolivier, L., Merrien, A. and Heulin, T. 2000. The role of *Rhizobium* sp. rhizobacterium that produces exopolysaccharide in the aggregation of the rhizospherical soil of the sunflower: Effects on plant growth and resistance to hydric constraint. *OCL – Oleagineux Corps Gras Lipides*, 6:524–528

Alloway, B. J. 2008. Zinc in soils and crop nutrition. Paris, France: IFA; and Brussels, Belg.

Aneja K. R. 2003. Experiments in Microbiology Plant Pathology and Biotechnology, 4th edition, New Age International Publishers, New Delhi, India

Ansary, M. H., Rahmani, H. A., Ardakani, M. R., Paknejad, F., Habibi, D. and Mafakheri, S. 2012. Effect of *Pseudomonas fluorescent* on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Annals of Biological Research*, 32: 1054-1062

Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R. and Lalande, R. 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil,* 204:57-68

Ardakani, S. S., Heydari, A., Khorasani, N. and Arjmandi, R. 2010. Development of new bioformulations of *Pseudomonas fluorescens* and evaluation of these products against damping-off of cotton seedlings. *Journal of Plant Pathology*, 83-88

Ardakani, S.S., Heydari, A., Tayebi, L. and Mohammadi, M. 2010. Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. *International Journal of Botany*, 62: 95-100

Arkhipova, T. N., Prinsen, E., Veselov, S. U., Martinenko, E. V., Melentiev, A. I. and Kudoyarova, G. R. 2007. Cytokinin producing bacteria enhance plant growth in drying soil. *Plant Soil*, 292: 305–315

Armada, E., Roldan, A. and Azcon, R. 2014. Differential activity of autochthonous bacteria in controlling drought stress in native Lavandula and Salvia plants species under drought conditions in natural arid soil. *Microbial Ecology*, 672: 410-420

Arshad, M., Shaharoona, B. and Mahmood T. 2008. Inoculation with plant growth promoting rhizo-bacteria containing ACC-deaminase partially eliminates the effects of water stress on growth, yield and ripening of *Pisum sativum* L. *Pedosphere,* 18:611–620

Arslan, M., Afzal, M., Amin, I., Iqbal, S. and Khan, Q. M. 2014. Nutrients can enhance the abundance and expression of alkane hydroxylase CYP153 gene in the rhizosphere of rye grass planted in hydrocarbon-polluted soil. *PloS one*, 910: e111208

Asghar, H., Zahir, Z., Arshad, M. and Khaliq, A. 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. *Biology and Fertility of Soils*, 354: 231-237

Ashraf, M. 2010. Inducing drought tolerance in plants: Recent advances. *Biotechnology Advances*, 28: 169-183

Bahadur, I., Meena, V. S. and Kumar, S. 2014. Importance and application of potassic biofertilizer in Indian agriculture. *International Research Journal of Biological Sciences,* 3:80–85

Bakker, A. W. and Schippers, B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. *Soil Biology and Biochemistry*, 194: 451-457

Baligh, M., Delgado, M. A. and Conway, K. E. 1999. Evaluation of *Burkholderia cepacia* strains: root colonization of *Catharanthus roseus* and *in vitro* inhibition of selected soil borne fungal pathogen. In: Proceedings of Oklahoma Academic Science, 79:19-27

Bastian, F., Cohen, A., Piccoli, P., Luna, V., Baraldi, R. and Bottini, R. 1998. Production of indole-3-acetic acid and gibberellins A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media. *Plant Growth Regulation*, 24: 7–11

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 454-493

Belimov, A. A., Dodd, I. C., Hontzeas, N., Theobald, J. C., Safronova, V. I., and Davies, W. J. 2009. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytologist*, 1812: 413-423

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. and Courchamp, F. 2012. Impacts of climate change on the future of biodiversity. *Ecology Letters*, 154: 365–377

Beniziri, E., Courtade, A., Picard, C. and Guckert, A. 1998. Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens* M.3.1. *Soil Biology and Biochemistry*, 3010-11: 1481-1484

Bensalim, S., Nowak, J. and Asiedu, S.K. 1998. A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. *American Journal of Potato Reearch*, 753:145–152

Bent, E., Tuzun, S., Chanway, C. P. and Enebak, S. 2001. Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Canadian Journal of Microbiology*, 479: 793-800

Bergey, David. H. 1994. *Bergey's Manual of Determinative Bacteriology* 9th ed., Lippincott Williams and Wilkins.

Bhaskar, P. V. and Bhosle, N. B. 2005. Microbial extracellular polymeric substances in marine biogeochemical processes. *Current Science,* 88:45–53

Bhattacharyya, P. N. and Jha, D. K. 2012. Plant growth-promoting rhizobacteria PGPR: emergence in agriculture. *World Journal of Microbiology and Biotechnology*, 284:1327-1350

Bhupinder, S., Senthil, A. N., Singh, B. K. and Usha, K. 2005. Improving zinc efficiency of cereals under zinc deficiency. *Current Science,* 88: 36-44

Boiero, L., Perrig, D., Masciarelli, O., Penna, C., Cassán, F. and Luna, V. 2007. Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Applied Microbiology and Biotechnology*, 744: 874-880

Bou-Abdallah, F. 2010. The iron reoc and hydrolysis chemistry of the ferritins. *Biochimica et Biophysica Acta BBA-General Subjects*, 18008: 719-731

Bull, C. T., Shetty, K. G. and Subbarao, K. V. 2002. Interactions between Myxobacteria, plant pathogenic fungi, and biocontrol agents. *Plant Disease*, 868: 889-896

Cabuslay, G. S., Ito, O. and Alejal, A. A. 2002. Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. *Plant Science,* 1634: 815-827

Cacciari, I., Lippi, D., Pietrosanti, T. and Pietrosanti, W. 1989. Phytohormone-like substances produced by single and mixed diazotrophic cultures of *Azospirillum* and *Arthrobacter*. *Plant Soil*, 115: 151–153

Cardoso, E. J. B. N. and Freitas, S. S. 1992. A rizosfera. In: Cardoso EJBN, Tsai SM, Neves PCP (eds) Microbiologia do solo. Sociedade Brasileira de Ciencia do Solo, Campinas, 41–57

Castillo, P., Escalante, M., Gallardo, M., Alemano, S., and Abdala, G. 2013. Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress. *Acta Physiologiae Plantarum*, 35: 2299–2309

Castric, P. A. 1974. "Hydrogen cyanide, a secondary metabolite of *Pseudomonas aeruginosa*" *Canadian Journal of Microbiology,* 21: 613-618

Chaiharn, M., Chunhaleuchanon, S., Kozo, A. and Lumyong, S. 2008. Screening of rhizobacteria for their plant growth promoting activities. *KMITL Sci. Tech. J.,* 8: 18-23

Chaves, M. M. and Oliveira, M. M. 2004. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. *Journal of Experimental Botany*, 55: 2365-2384

Chen, C., Belanger, R. R., Benhamou, N. and Paullitz, T. C. 2000. Defence enzymes induced in cucumber roots by treatment with plant-growth promoting rhizobacteria PGPR. *Physiological and Molecular Plant Pathology,* 56:13–23

Chen, C., Xin, K., Liu, H., Cheng, J., Shen, X., Wang, Y., and Zhang, L. 2017. *Pantoea alhagi*, a novel endophytic bacterium with ability to improve growth and drought tolerance in wheat. *Scientific Reports*, 7:41564

Chenu, C. 1993. Clay or sandpolysaccharide associations as models for the interface between micro-organisms and soil: water related properties and microstructure. In *Soil Structure/Soil Biota Interrelationships*. 143-156

Chenu, C. and Roberson, E. B. 1996. Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biology and Biochemistry*, 28:877–884

Chiarini, L., Bevivino, A., Tabacchioni, S. and Dalmastri, C. 1998. Inoculation of *Burkholderia cepacia, Pseudomonas fluorescens* and *Enterobacter* sp. on Sorghum bicolor: root colonization and plant growth promotion of dual strain inocula. *Soil Biology and Biochemistry*, 301: 81-87

Choure, K., and Dubey, R. C. 2012. Development of plant growth promoting microbial consortium based on interaction studies to reduce wilt incidence in *Cajanus cajan* L. var. Manak. *World Journal of Agricultural Sciences*, 81: 118-128

Chowdhury, S, P., Schmid, M., Hartmann, A. and Tripathi, A. K. 2007. Identification of diazotrophs in the culturable bacterial community associated with roots of *Lasiurus sindicus* a perennial grass of thar desert, India. *Microbial Ecology*, 54: 82-90

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C. and Barka, E. A. 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology*, 714: 1685-1693

Correia, M.J., Coelho, D. and David, M.M. 2001. Response to seasonal drought in three cultivars of *Ceratonia siliqua*: leaf growth and water relation. *Tree Physiology,* 21: 645–653

Dave, A. and Patel, H. H. 1999.Inorganic phosphate solubilizing soil Pseudomonads. *Indian Journal of Microbiology*, 393: 161-164

De Meyer, G., Capieau, K., Audenaert, K., Buchala, A., Metraux, J.P. and Hofte, M. 1999. Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. *Molecular Plant-Microbe Interactions,* 12: 450–458

De Vleesschauwer, D., Djavaheri, M., Bakker, P. A. and Hofte, M. 2008. *Pseudomonas fluorescens* WCS374r-induced systemic resistance in rice against *Magnaporthe oryzae* is based on pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. *Plant physiology*, 1484: 1996-2012.

Deepak, J., Geeta, N., Sachin, V. and Anita, S. 2013. Enhancement of wheat growth and Zn content in grains by zinc solubilizing bacteria. *International Journal* of *Agriculture Environment* and *Biotechnology*, 6: 363–370

Defago, G., Berling, C.H., Burger, U., Haas, D., Kahr, G., Keel, C., Voisard, C., Wirthner, P. and Wuthrich, B. 1990. Suppression of black root rot of tobacco and other root diseases by strains of *Pseudomonas fluorescens* potential applications and mechanisms. In: Biological control of soil bore plant pathogens, D. Hornby Ed. CAB International, Walligfort, Oxon, U.K. 93-98

DelloIoio, R., Linhares, F, S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P. and Sabatini, S. 2007. Cytokinins determine Arabidopsis root-meristem size by controlling cell differentiation. *Current Biology*, 17: 678-682

Delrio, L. A., Corpas, F. J., Sandalio, L. M., Palma, J. M. and Barroso, J. B. 2003. Plant peroxisomes, reactive oxygen metabolism and nitric oxide. *IUBMB life*, 552:71-81

Desai, S., Kumar, P., Sultana, U., Pinisetty, S. and Reddy, G. 2012. Potential microbial candidate strains for management of nutrient requirements of crops. *African Journal of Microbiology Research*, 617:3924-3931

Dimkpa, C. O., Merten, D., Svatos, A., Buchel, G. and Kothe, E. 2009. Siderophores mediate reduced and increased uptake of cadmium by *Streptomyces tendae* F4 and sunflower *Helianthus annuus*, respectively. *Journal Applied Microbiology,* 107: 1687–1696

Dimkpa, C., Weinand, T. and Asch, F. 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment*, 3212: 1682-1694

Din, J., Khan, S.U., Ali, I. and Gurmani, A.R. 2011. Physiological and agronomic response of canola varieties to drought stress. *Journal* of *Animal and Plant Sciences*, 21:78–82

Dobbelaere, S., Vanderleyden, J. and Okon, Y. 2003. Plant growthpromoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences,* 22:107-149

Drabble, E. and Drabble, H. 1907. The relation between the osmotic strength of cell sap in plants and their physical environment. *Biochemical Journal*, 2: 117–132

Duffus, C. M. 1992. Control of starch biosynthesis in developing cereal grains. *Biochemical Society Transactions,* 20: 13–18

Etesami, E., Alikhani, H. A. and Akbari, A. A. 2009. Evaluation of Plant growth hormones production IAA ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth indexes. *World Applied Science Journal,* 611: 1576-1584

Figueiredo, M. V. B., Burity, H. A., Martinez, C. R. and Chanway, C. P. 2008. Alleviation of drought stress in the common bean *Phaseolus vulgaris* L. by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Applied Soil Ecology*, 40:182–188

Flemming, H. and Wingender, J. 2001. Relevance of microbial extracellular polymeric substances EPSs: part I. Structural and ecological aspects. *Water Science* and *Technology,* 43: 1–8

Gangwar, M. and Kaur, G. 2009. Isolation and characterization of endophytic bacteria from endorhizosphere of sugarcane and rye grass. *Internet Journal of Microbiology,* 7:139–144

Gaur, A. C. 1990. Phosphate Solubilizing Microorganisms as Biofertilizers. Omega Scientific Publishers, New Delhi. 176

Gerretsen, F. C. 1948. The influence of microorganisms on the phosphorus uptake by the plant. *Plant and Soil*, 1: 51-81

Glick, B. R. 1995. The enhancement of plant growth by free-living bacteria. *Canadian journal of Microbiology*, 41: 109-117

Glick, B. R. 2012. Plant Growth-Promoting Bacteria: Mechanisms and Applications; Hindawi Publishing Corporation, Scientifica: Waterloo, Canada.

Glick, B. R., Karaturovic, D. M. and Newell, P. C. 1995. A novel procedure for rapid isolation of plant growth promoting *Pseudomonads*. *Canadian Journal of Microbiology*, 416: 533-536

Goenadi, D. H. and Sugiarto, Y. 2000. Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. *Soil science society of America journal*, 64(3): 927-932

Gordon, S. A. and Weber R. P. 1951. Colorimetric estimation of indole-acetic acid. *Plant Physiol*ogy, 26:192-195

Gravel, V., Antoun, H. and Tweddell, R. J. 2007. Growth stimulation and fruit yield of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: Possible role of indole acetic acid IAA. *Soil Biology and Biochemistry,* 39: 1968-1977

Gray, E. J. and Smith, D. L. 2005. Intracellular and extracellular PGPR: Commonalities and distinctions in the plant- bacterium signaling processes. *Soil Biology and Biochemistry,* 37, 395-412

Grover, M., Ali, S. Z., Sandhya, V., Rasul, A. and Venkateswarlu, B. 2010. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World Journal of Microbiology and Biotechnology*, 275: 1231-1240

Guetsky, R., Shtienberg, D., Elad, Y., Fischer, E. and Dinoor, A. 2002. Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. *Phytopathol*, 92: 976-985

Gururani, M. A., Upadhyaya, C. P., Strasser, R. J., Yu, J. W. and Park, S. W. 2013. Evaluation of abiotic stress tolerance in transgenic potato plants with reduced expression of PSII manganese stabilizing protein. *Plant Science*, 198: 7-16

Gusain, Y. S., Singh, U. S. and Sharma, A. K. 2015. Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, 149: 764-773

Gutterson, N., Layton, T. J. and Warren, G. J. 1986. Molecular cloning of genetic determinants for inhibition of fungal growth by a fluorescent pseudomonad. *Journal of Bacteriology*, 165: 696-703

Haas, D. and Defago, G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nature Reviews Microbiology*, 34: 307

Halbleib, C. M. and Ludden, P. W. 2000. Regulation of biological nitrogen fixation. *The Journal of Nutrition*, 1305: 1081-1084

Hamayun, M., Khan, S. A., Shinwari, Z. K., Khan, A. L., Ahmad, N. and Lee, I. J. 2010. Effect of polyethylene glycol induced drought stress on physio-hormonal attributes of soybean. *Pakistan Journal of Botany,* 422: 977-986

Han, J., Sun, L., Dong, X., Cai, Z., Sun, X., Yang, H. and Song, W. 2005. Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. *Systematic and Applied Microbiology*, 281: 66-76

Handelsman, J. and Stabb, E. V. 1996. Biocontrol of soilborne plant pathogens. *Plant Cell,* 8: 1855-1869

Hankin, L. and Anagnostakis, S. L. 1975. The use of solid media for detection of enzyme production by fungi. *Mycologia*, 597-607

Hayat, Q., Hayat, S., Irfan, M. and Ahmad, A. 2010. Effects of exogenous salicyclic cid under changing environment: A review. *Environmental and Experimental Botany,* 68: 14-25

Hayat, S., Hayat, Q., Alyemeni, M. N., Wani, A. S., Pichtel, J. and Ahmad, A. 2012. Role of proline under changing environments. *Plant Signaling and Behavior*, 7: 1456–1466

Heath, R. L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics*, 1251: 189-198

Heidari, M. and Golpayegani, A. 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria PGPR on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *Journal of the Saudi Society* of *Agricultural Sciences,* 11 1: 57-61

Heijnen, C. E., Burgers, S. L. G. E. and Van Veen, J. A. 1993. Metabolic activity and population dynamics of rhizobia introduced into unamended and bentonite-amended loamy sand. *Applied and Environmental Microbiology*, 593: 743-747

Hill, D. S., Stein, J. I., Torkewitz, N. R., Morse, A. M., Howell, C. R., Pachla-tko, J. P., Becker, J. O. and Ligon, J. M. 1994. Cloning of genes involved in the synthesis of pyrrolnitrin from *Pseudomnas fluorescens* and role of pyrrolnitrin synthesis in biological control of plant disease. *Applied and Environmental Microbiology,* 60:78-85

Hiscox, J. T. and Israelstam, G. F. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany*, 5712: 1332-1334

Honma, M. and Shimomura, T. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agricultural and Biological Chemistry*, 4210:1825-1831

Huang, B., DaCosta, M. and Jiang, Y. 2014. Research advances in mechanisms of turf grass tolerance to abiotic stresses: from physiology to molecular biology. *Critical Reviews in Plant Sciences*, 33: 141–189

Hunter, R. C. and Beveridge, T. J. 2005. High resolution visualization of *Pseudomonas aeruginosa* PAO1 biofilms by freeze substitutions electron microscopy. *Journal of Bacterioogy*, 187:7619–7630

Hussain, A., Arshad, M., Zahir, Z. A. and Asghar, M. 2015. Prospects of zinc solubilizing bacteria for enhancing growth of maize. *Pakistan Journal* of *Agricultural Sciences,* 52: 915–922

Illmer, P. and Schinner, F. 1992. Solubilization of inorganic phosphates by microorganisms isolated from forest soils. *Soil Biology and Biochemistry*, 244: 389-395

IPCC. 2007. Fourth assessment report: synthesis. Published online 17 november 2007 www.ipcc.ch/ipccreports/ar4-syr.htm.

Jarvis, P. G. and Jarvis, M. S. 1963. The water relations of tree seedlings. IV. Some aspects of the tissue water relations and drought resistance. *Physiologia Plantarum*, 16: 501–516

Jat, L. K., Singh, Y. V., Meena, S. K., Meena, S. K., Parihar, M., Jatav, H. S., Meena, R. K. and Meena, V. S. 2015. Does integrated nutrient management enhance agricultural productivity? *Journal of Pure and Applied Microbiology,* 92:1211–1221

Jha, C. K. and Saraf, M. 2012. Evaluation of Multispecies Plant-Growth-Promoting Consortia for the Growth Promotion of *Jatropha curcas* L. *Journal of plant growth regulation*, 31(4): 588-598

Jha, Y. and Subramanian, R. B. 2016. Regulation of plant physiology and antioxidant enzymes for alleviating salinity stress by potassium-mobilizing bacteria. In: Meena, V.S., Maurya, B.R., Verma, J.P., Meena, R.S. eds Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, 149–162

Jorjani, M., Heydari, A., Zamanizadeh, H., Rezaee, S. and Naraghi, L. 2011. Development of *Pseudomonas fluorescens* and *Bacillus coagulans* based bio formulations using organic and inorganic carriers and evaluation of their influence on growth parameters of sugar beet. *Journal of Bio Pesticides*, 4(1): 180-185

Kabata-Pendias, A. and Pendias, H. 2001. Trace elements in soils and plants. CRC Press, Boca Raton, FL 3rd edition, 413

Kakar, K. U., Ren, X. L., Nawaz, Z., Cui, Z. Q., Li, B., Xie, G. L. and Sun, G. C. 2016. A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). *Plant Biology*, 183: 471-483

Kamara, A.Y., Menkir, A., Badu-Apraku, B. and Ibikunle, O. 2003. The influence of drought stress on growth, yield and yield components of selected maize genotypes. *Journal of Agricultural Science***,** 1411:43

Kandpal, R. P., Vaidyanathan, C. S., Kumar, M. U. and Sastry, K. S. 1981. Alterations in the activities of the enzymes of proline metabolism in Ragi *Eleusine coracana* leaves during water stress *Journal of Biological Science,* 3: 361-370

Karnwal, A. 2009.Production of Indol acetic acid by fluorescent Pseudomonas in the presence of L-Tryptophan and Rice root exudates. *Journal of Plant Pathology*, 91: 61-63

Karunanithi, K., Muthusamy, M. and Seetharaman, K. 2001. Gypsum-A suitable material for the mass multiplication of *Trichoderma viride. Tropical Agricultural Research and Extension*, 42: 115-116

Kasana, R.C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. 2008. A rapid and easy method for the detection of microbial cellulases on agar plates using Gram's iodine. *Current Microbiology*, 575: 503-507

Katiyar, V. and Goel, R. **2004**. Siderophore-mediated plant growth promotion at low temperature by mutant of fluorescent pseudomonad. *Plant Growth Regulation,* 42: 239–244

Kavino, M., Harish, S., Kumar, N., Saravanakumar, D. and Samiyappan, R. 2008. Induction of systemic resistance in banana Musa spp. against Banana bunchy top virus BBTV by combining chitin with root-colonizing *Pseudomonas fluorescens* strain CHAO. *European Journal of Plant Pathology*, 120: 353–362

Kemble, A. R. and Macpherson, H. T. 1954. Liberation of amino acids in perennial rye grass during wilting. *Biochemical Journal,* 58: 46–49

Keyvan S. 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *Journal of Animal and Plant Sciences,* 83:1051-1060

Khalid, A., Tahir, S., Arshad, M. and Zahir, Z. A. 2004. Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. *Soil Research*, 428: 921-926

Khan, A. 2017. Characterization of drought tolerant plant growth promoting rhozobacteria and their evaluation on wheat. Thesis, M.Sc. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar

Khan, M. S., Zaidi, A., Wani, P. A. and Oves, M. 2009. Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, 71:1-19

Kiiskinen L. L, Ratto M, Kruus K 2004. Screening for novel laccase producing microbes. *Journal of Applied Microbiology*, 97: 640-646

Kim, J. and Rees, D. C. 1994. Nitrogenase and Biological Nitrogen Fixation. *Biochemistry*, 33: 389-397

Kirk, J. T. O. and Allen, R. L. 1965. Dependence of chloroplast pigment synthesis on protein synthesis: effect of actidione. *Biochemicaland Biophysical Research Communications*, 216: 523-530

Kloepper, J. W., Leong, J., Teintze, M. and Schroth, M. N. 1980. Enhancing plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, 286: 885–886

Kloepper, J. W., Ryu, C. M. and Zhang, S. A. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology,* 94:1259-1266

Kobayashi, D. and El-Barrad, N. H. 1996. Selection of bacterial antagonists using enrichment cultures for the control of summer patch disease in kentucky bluegrass. *Current Microbiology,* 32: 106-110

Kobayashi, D. Y., Reedy, R. M., Bick, J. A, Oudemans P. V. 2002. Characterization of chitinase genefrom *Stenotrophomonas maltophilia*strain 34S1 and its involvement in biological control. *Applied and Environmental Microbiology,* 683:1047–1054

Krugman, T., Peleg, Z., Quansah, L., Chague, V., Korol, A. B., Nevo, E., Saranga, Y., Fait, A., Chalhoub, B. and Fahima, T. 2011. Alteration in expression of hormone-related genes in wild emmer wheat roots associated with drought adaptation mechanisms. *Functional and Integrative Genomics,* 11: 565–583

Kumar, A., Bahadur, I., Maurya, B. R., Raghuwanshi, R., Meena, V. S., Singh, D. K. and Dixit, J. 2015. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability? *Journal of Pure and Applied Microbiology*, 9:715–724

Kumar, A., Patel, J. S., Bahadur, I. and Meena, V. S. 2016. The molecular mechanisms of KSMs for enhancement of crop production under organic farming. In: Meena, V. S., Maurya, B. R., Verma, J. P. and Meena R. S eds Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, 61–75

Kumar, S. 2015. Isolation and characterization of plant growth promoting rhizobacteria for biofortification of iron and zinc in wheat. Thesis, M.Sc. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar

Kumar, S., Kumar, R. and Om, H. 2013. Shelf-life of *Trichoderma viride* in talc and charcoal based formulations. *Indian Journal of Agricultural Sciences*, 835: 566-9

Ladha, J. K., Kirk, G. J. D., Bennett, J., Reddy, C. K., Reddy, P. M. and Singh, U. 1998. Opportunities for increased nitrogen use efficiency from improved lowland rice germplasm. *Field Crops Research*, 56:41-71

Lafitte, H. R., Guan., Y. S. Shi, Y. and Li, Z. K. 2007. Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *Journal of Experimental Botany*, 58: 169-175

Lesk, C., Rowhani, P. and Ramankutty, N. 2016. Influence of extreme weather disasters on global crop production. *Nature,* 529:84–87

Liu, N., Liu, Y., Bao, G., Bao, M., Wang, Y., Zhang, Ge. Y., Bao, W. and Tain, H. 2016. Drought reconstruction in eastern hulun buir steppe, china and its linkage to the sea surface temperatures in the Pacific Ocean, *Journal of Asian Earth Sciences*, 115: 298-307

Lu, Y., Zhang, S., Shah, T., Xie, Chuanxiao., Hao, Z., Li, Xinhai., Farkhari, M., Ribaut, J. M., Cao, M., Rong, T. and Xu, Y. 2010. Joint linkage –linkage disequilibrium mapping is a powerful approach of detecting quantitative trait loci underlying drought tolerance in maize. *Proceedings of the National Academy of Sciences,* 107: 19585-19590

Luck, H. 1974. Methods of enzymatic analysis. Academic Press. 885-894

Lutgtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. *Annual* Review of *Microbiology*, 63: 541-556

Madigan, M. T. and Martinko, J. M. 2006. Brock biology of microorganisms. XI edition. Pearson Education international

Maksimov, I. V., Abizgil'Dina, R. R. and Pusenkova, L. I. 2011. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens. *Applied Biochemistry and Microbiology*, 474: 333-345

Manca de Nadra, M. C., Strasser de Saad, A. M. Pesce de Ruiz Holgado, A. and Oliver, G. 1985. Extracellular polysaccharide production by *Lactobacillus bulgaricus* CRL 420 *Milchwissenschaft*, 40: 409-411

Maurhofer, M., Hase, C., Meuwly, P., Metraux, J. P. and Defago, G. 1994. Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: Influence of the gacA gene and of pyoverdine production. *Phytopathology USA*

Maurya, B. R., Meena, V. S. and Meena, O. P. 2014. Influence of Inceptisol and Alfiol's potassium solubilizing bacteria KSB isolates on release of K from waste mica. *Vegetos*, 27:181–187

Mayak, S., Tirosh, T. and Glick, B. R. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiology and Biochemistry*, 426: 565-572

McKenzie, R. H. and Roberts, T. L. 1990. Soil and fertilizers phosphorus update. In: Proceedings of Alberta Soil Science Workshop Proceedings. *Edmonton Alberta*, 84-104

Meena, V. S., Maurya, B. R. and Meena, R. S. 2015. Residual impact of well grow formulation and NPK on growth and yield of wheat (*Triticum aestivum* L.). *Bangladesh Journal of Botany,* 441:143–146

Megala, S. and Paranthaman. 2017. Effect on the plant growth promoting rhizobacteria PGPR increasing plant height, chlorophyll and protein content of *Solanum nigrum*. *International Journal of Applied Research*, 312: 147-150

Michel, B. E. and Kaufmann, M. R. 1973. The osmotic potential of polyethylene glycol 6000. *Plant Physiol*. 515: 914-916

Minamisawa, K., Nishioka, K., Miyaki, T., Ye, B., Miyamoto, T., You, M., Saito, A., Saito, M., Bamaquio, W. L., Teaumroong, N., Sein, T. and Sat, T. 2004. Anaerobic nitrogen-fixing consortia consisting of clostridia isolated from gramineous plants. *Applied and Environmental Microbiology*, 70: 3096-3102

Mishra, J., Tewari, S., Singh, S. and Arora, N. K. 2015. Biopesticides: where we stand? In *Plant Microbes Symbiosis: Applied Facets,* 37-75. Springer, New Delhi

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in plant science*, 79: 405-410

Modi, B., Bindra, M. and Modi, V. 1989. "Extracellular polysaccharides of Cowpeas Rhizobia; Compositional and functional studies" *Archieve in Microbiology*, 1: 1-15

Mohite, B. 2013. Isolation and characterization of indole acetic acid IAA producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*, 133: 638-649

Moran, J. F., Becan, M., Iturbe-Ormaetyl, I., Frechilla, S., Klucas, R. V. and AparicioTejo, P. 1994. Drought induces oxidative stresses in pea plants. *Planta*, 194: 346–352

Morris, M. L., Belaid, A. and Byerlee, D. 1991. Wheat and barley production in rainfed marginal environments of the developing world. *In*: CIMMYT World Wheat Facts and Trends, 1990-1991. CIMMYT, Mexico

Nagoba, B. and Vedpathak, D. 2011. Medical applications of siderophores. *European Journal of General Medicine*, 8: 229–235

Nain L., Rana A., Joshi M., Shrikrishna J. D., Kumar D., Shivay Y. S., Paul S. and Prasanna R. 2010. Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. *Plant Soil*, 331: 217–230

Nair, A., Abraham, T. K. and Jaya, D. S. 2008. Studies on the changes in lipid peroxidation and antioxidants in drought stress induced Cowpea *Vigna unguiculata* L. varieties. *Journal of Environmental Biology*, 29: 689-691

Naz, I., Ahmad, H., Khokhar, S. N., Khan, K., and Shah, A. H. 2016. Impact of zinc solubilizing bacteria on zinc contents of wheat. *American Eurasian Journal of Agriculture and Environmental Sciences*, 16: 449–454

Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J. L. and Thonart, P. 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environmental Microbiology*, 94: 1084-1090

Ordentlich, A., Elad, Y. and Chet, I. 1988. The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. *Phytopathology*, 78: 84-88

Oren, R., Sperry J. S, Katul, G. G Pataki, D. E., Ewers, B. E., Phillips, N. and Schafer, K. V. R. 1999. Survey and synthesis of intra and inter specific variation of stomatal sensitivity to vapour pressure deficit. *Plant Cell Environment*, 22: 1515–26

Pandey, A., Trivedi, P., Kumar, B. and Palni, L. M. S. 2006. Characterization of a phosphate solubilizing and antagonistic strain of *Pseudomonas putida* B0 isolated from a sub-alpine location in the Indian Central Himalaya. *Current Microbiology*, 532: 102-107

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S. and Sa, T. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. *Microbiological Research*, 1602: 127-133

Patten, C. L. and Glick, B. R. 1996. Bacterial biosynthesis of indole-3-acetic acid. *Canadian Journal of Microbiology*, 423: 207-220

Patten, C. L. and Glick, B. R. 2002. Role of *Pseudomonas putida* indole acetic acid in development of the host plant root system. *Applied and Environmental Microbiology*, 688:3795-3801

Paul, M. J., Primavesi, L. F., Jhurreea, D. and Zhang, Y. 2008. Trehalose metabolism and signaling. *Annual Review of Plant Biology*, 59: 417–44

Payne, S. M. 1994. *Methods Enzymol*. 235:329

Pinedo, I., Ledger, T., Greve, M. and Poupin, M. J. 2015. *Burkholderia phytofimans* PsJN induces long-term metabolic and transcriptional changes involved in *Arabidopsis thaliana* salt tolerance. *Frontier Plant Science,* 6466: 1-17.

Podile, A. R. and Kishore, G. K. 2007. Plant growth-promoting rhizobacteria. In *Plant-associated bacteria*. 195-230. Springer, Dordrecht.

Qi, W. Z. and Zhao, L. 2013. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. *Journal of Basic Microbiology,* 53: 355–364

Qureshi, M. A., Shakir, M. A., Iqbal, A., Akhtar N., Khan A. 2012. Co-inoculation of phosphate solubilizing bacteria and rhizobia for improving growth and yield of mungbean (*Vigna radiata* L.). *Journal of Animal Plant Science,* 21: 491–497

Rajasekar, S. and R. Elango, 2011. Effect of microbial consortium on plant growth and improvement of alkaloid content in (*Withania somnifera*) Ashwagandha. *Current Botany*, 28: 27-30

Rajkumar, M., Ae, N., Prasad, M. N. V. and Freitas, H. 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. *Trends in Biotechnology*, 283: 142-149

Ramesh, A., Sharma, S. K., Sharma, M. P., Yadav, N. and Joshi, O. P. 2014. Inoculation of zinc solubilizing *Bacillus aryabhattai* strains for improved growth, mobilization and biofortification of zinc in soybean and wheat cultivated in Vertisols of central India. *Applied Soil Ecology,* 73: 87-96

Rampino, P., Pataleo, S., Gerardi, C., Mita, G. and Perrotta, C. 2006. Drought stress response in wheat: physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ*, 2912: 2143-2152

Rana, A., Joshi, M., Prasanna, R., Shivay, Y. S. and Nain, L. 2012. Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *European Journal of Soil Biology*, 50: 118-126

Raval, A. A., and P. B. Desai. 2012. Rhizobacteria from rhizosphere of sunflower *Helianthus annuus* L. and their effect on plant growth. *Research Journal of Recent Sciences*, 1:58-61

Rawat, S. 2016. Zinc and Iron Biofortification Strategies through Plant Growth Promoting Rhizobacteria in Wheat. Thesis, M.Sc. Govind Ballabh Pant University of Agriculture and Technology, Pantnagar.

Richardson, A. E., Barea, J. M., McNeill, A. M. and Prigent-Combaret, C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil,* 321: 305–339

Riggs, P. J., Chelius, M. K., Iniguez, A. L., Kaeppler, S. M. and Triplett, E.W. 2001. Enhanced maize productivity with diazotrophic bacteria. *Australian Journal of Plant Physiology*, 28:829-836

Roberson, E. B. and Firestone, M. K. 1992. Relationship between desiccation and exopolysaccharide production in soil *Pseudomonas* sp. *Applied and Environmental Microbiology*, 58:1284–1291

Rodriguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances,* 17: 319-339

Rogers, M. B., Sexton, J. A., DeCastro, G. J. and Calderwood, S. B. 2000. Identification of an operon required for ferrichrome iron utilization in *Vibrio cholerae*. *Journal of Bacteriology*, 182(8): 2350-2353

Ryan R. P., Monchy S., Cardinale M., Taghavi S., Crossman L., Avison M. B. 2009. The versatility and adaptation of bacteria from the genus Stenotrophomonas. *Nature Reviews,* 7: 514–525

Sairam, R. K., Srivastava, G. C., Agarwal, S. and Meena, R. C. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biologia Plantarum***,** 49: 85–91

Sandhya, V., Ali, S. Z., Grover, M., Reddy, G. and Venkateswarlu, B. 2009. Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biology and Fertility of Soils*, 461: 17-26

Sandhya, V., Ali, S. Z., Grover, M., Reddy, G. and Venkateswarlu, B. 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regulation*, 621: 21-30

Sandikar, B. M. and Awasthi, R. S. 2009. Preparation and shelf-life study of *Pseudomonas* and *Bacillus* bioformulations against phytopathogenic *Pythium* and *Fusarium* species. *International Journal of Plant Protection*, 22: 251-254

Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P. and Samiyappan, R. 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiologiae Plantarum*, 331: 203-209

Sasirekha, B. and Srividya, S. 2016. Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. *Agriculture and Natural Resources*, 50(4): 250-256

Schwyn, B. and Neilands, J. B. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry*, 1601: 47-56

Shahraki, M., Heydari, A. and Hassanzadeh, N. 2009. Investigation of antibiotic, siderophore and volatile metabolite production by bacterial antagonists against *Rhizoctonia solani. Iranian Journal of Biology*, 22 (1): 71–84

Shaikh, S. S. and Sayyed, R. Z. 2015. Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In *Plant Microbes Symbiosis: Applied Facets*, 337-351, Springer, New Delhi

Shaikh, S. S., Sayyed, R. Z. and Reddy, M. S. 2016. Plant Growth-Promoting Rhizobacteria: An Eco-friendly Approach for Sustainable Agroecosystem. In *Plant, Soil and Microbe s*pp.181-201. Springer, Cham.

Shakir, M. A., Asghari, B. and Arshad, M. 2012. Rhizosphere bacteria containing ACC deaminase conferred drought tolerance in wheat grown under semi-arid climate. *Soil and Environment*, 31:108–12

Sharma, R. C., Sarkar, S., Das, D. and Banik, P. 2013. Impact assessment of arbuscular mycorrhiza *Azospirillum* and chemical fertilizer application on soil health and ecology. *Communications in Soil Science and Plant Analysis*, 44(6): 1116-1126

Sheffield, J., Wood, E. F., Chaney, Nathaniel., Guan, K., Sadri, S. and Yaun, X. 2014. A Drought monitoring and forecasting system for sub-Sahara African water resources and food security. *Bulletin of the American Meteorological Society*, 95: 861 –882

Shenoy, V. V. and Kalagudi, G. M. 2003. Meta-bug and near-isogenic strainconsortia concepts for plant growth promoting rhizobacteria. In: 6th International PGPR workshop, India, section VII—mechanism of biocontrol. 108

Shintu, P. V. and Jayaram, K. M. 2015. Phosphate solubilising bacteria *Bacillus polymyxa*-An effective approach to mitigate drought in tomato *Lycopersicon esculentum* Mill. *Tropical Plant Research*, 2: 17-22

Singh, A. V., Chandra R. and Goel R. 2013. Phosphate solubilization by *Chryseobacterium* sp. and their combined effect with N and P fertilizers on plant growth promotion. *Archives of Agronomy and Soil Science,* 59(5): 641– 651.

Singh, A. V., Prasad B. and Shah, S. 2010. Screening plant growth promotory rhizobacteria for improving seed germination and seedling vigor of lentil *Lens culinaris* Medik. *Environment and Ecology,* 28 (3B):2055-2058

Singh, A., Mishra, A. K., Singh, S. S., Sarma, H. K. and Shukla, E. 2008. Influence of iron and chelator on siderophore production in *Frankia* strains nodulating *Hippophae salicifolia* D. Don. *Journal of Basic Microbiology*, 482: 104-111

Singh, J. and Singh, A. V. 2017. Microbial Strategies for Enhanced Phytoremediation of Heavy Metals Contaminated Soils. Environmental Pollutants and their Bioremediation Approaches. *Taylor and Francis*, 9: 249-264

Singh, J., Singh, A. V., Prasad, B. and Shah, S. 2017. Sustainable agriculture strategies of wheat biofortification through microorganisms. Wheat A premier Food Crop. 374-391

Socias, F. X, . Correla, M. J. and Medrano, H. 1997. The role of abscisic acid and water relations in drought responses of substerranean clover. *Journal of Experimental Botany*, 48: 1281- 1288

Stefan, M., Munteanu, N., Stoleru, V. and Mihasan, M. 2013. Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean. *Romanian Biotechnological Letters*, 18: 8132- 8143

Stockwell, V. O., Johnson, K. B., Sugar, D. and Loper, J. E. 2011. Mechanistically compatible mixtures of bacterial antagonists improve biological control of fire blight of pear. *Phytopathology,* 101: 113- 123

Strzelczyk, E., Kamper, M. and Li, C. 1994. Cytokinin-like-substances and ethylene production by *Azospirillum* in media with different carbon sources. *Microbiological Research*, 149:55–60

Susilowati, A., Puspita, A. A. and Yunus, A. 2018. Drought resistant of bacteria producing exopolysaccharide and IAA in rhizosphere of soybean plant *Glycine max* in Wonogiri Regency Central Java Indonesia. In *IOP Conference Series: Earth and Environmental Science*, 142(1):12-58

Thomas-Edison, E., Martin, D. C. J. and Torres, O. 2012. Gelatin hydrolysis test protocol library. *http://www.Microbeblibrary.ord/library/lLaboratorytest/.*

Tien, T. M., Gaskins, M. H. and Hubbell, D. H. 1979. Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet *Pennisetum americanum* L. *Applied and Environmental Microbiology,* 37: 1016-1024

Tiwari, S., Lata, C., Chauhan, P. S. and Nautiyal, C. S. 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiology and Biochemistry*, 99:108–117

Trevors, J. T. 1992. Use of alginate and other carries for encapsulation of microbial cells for use in soil. *Microbiological Research,* 1: 61-69

Tsavkelova, E. A., Cherdyntseva, T. A., Botina, S. G. and Netrusov, A. I. 2007. Bacteria associated with orchid roots and microbial production of auxin. *Microbiological Research*, 1621: 69-76

Upadhyay, H. and Panda, S. K. 2013. Abiotic stress responses in tea camellia sinesiso kuntze: An overview. *Reviews in Agricultural Sciences,* 1:1-10

Vaid, S. K., Kumar, B., Sharma, A., Shukla, A. K. and Srivastava, P. C. 2014. Effect of zinc solubilizing bacteria on growth promotion and zinc nutrition of rice. *Journal of Soil Science and Plant Nutrition*, 14: 889–910

Van Loon, L. C. 2007. Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology,* 119:243-254

Vanhooren, P. and Vandamme, E. J, 1998. Biosynthesis, physiological role, use and fermentation process characteristics of bacterial exopolysaccharides. *Recently published articles from Journal of Bioscience and Bioengineering,* 1: 253–299

Vardharajula, S. 2014. Exopolysaccharide production by drought tolerant bacillus spp. And effect on soil aggregation under drought stress. *The Journal of Microbiology, Biotechnology and Food Sciences*, 41-51

Venkatakrishnan, S. S., Sudalayandy, R. S. and Savariappan, A. R. 2003. Assessing in vitro solubilization potential of different zinc solubilizing bacterial ZSB strains. *Brazilian Journal of Microbiology*, 34: 121-125

Verma, J. P., Yadav, J., Tiwari, K. N. and Lavakush, S. V. 2010. Impact of plant growth promoting rhizobacteria on crop production. *International Journal of Agricultural Research,* 5(11): 954-983

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*, 255 (2): 571-586

Vidhyasekaran, P. and Muthamilan, M. 1995. Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Disease*, 79: 782-786

Vidhyasekaran, P. and Muthamilan, M. 1999. Evaluation of powder formulation of *Pseudomonas fluorescens* Pf1 for control of rice sheath blight. *Biocontrol Science Technology,* 9:67–74

Vinocur, B. and Altman, A. 2005. Recent Advances in Engineering Plant Tolerance to Abiotic Stress: Achievements and Limitations**.** *Current Opinion in Chemical Biology*, 16:123-132

Visca, P., Ciervo, A., Sanfilippo, V. and Orsi, N. 1993. Iron-regulated salicylate synthesis by *Pseudomonas* spp. *Journal of General Microbiology,* 139:1995–2001

Vora, M. S. and Shelat, H. N. 1996. Solubilization of inorganic phosphates by microorganisms isolated from Anand soil. *Madras Agricultural Journal,* 83: 353-354

Vurukonda, S. S., Vardharajula, S., Shrivastava, M. and SkZ, A. 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research,* 184:13–24

Wallace, J. S., Acreman, M. C. and Sullivan, C. A. 2003. The sharing of water between society and ecosystems: from conflict to catchment–based co–management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 358: 2011-2026

Wang, C. J., Yang, W., Wang, C., Gu, C., Niu, D. D., Liu, H. X., Wang, P.Y. and Guo, J. H. 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *PLoS One*, 712: 555-565

Wang, X., Wang, Y., Tian, J., Lim, B. L., Yan, X. and Liao, H. 2009. Overexpressing AtPAP15 enhances phosphorus efficiency in soybean. *Plant Physiology*, 151:233–240

Wetherley, P. 1950. Studies in the water relations of the cotton plant. *New Phytologist*, 491: 81-97

Whipps, J. M. 2001. Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany*, 52: 487-511

Wilhite, D. A. and Glantz, M. H. 1985. Understanding the drought phenomenon: the role of definitions. *Water International,* 10: 111–120

Wingender, J., Neu, T. R. and Flemming, H. C. 1999. What are bacterial extracellular polymeric substances?. In: *Microbial extracellular polymeric substances* 1-19. Springer, Berlin, Heidelberg.

Wu, D. X., Wang, G. X., Bai, Y. E. and Liao, J. X. 2004. Effects of elevated CO_2 concentration on growth, water use, yield and grain quality of wheat under two soil water levels. *Agriculture Ecosystem and Environment,* 104: 493-507

Yadav, K. and Singh, T. 1991. Phosphorus solubilization by microbial isolate from a Cacifluvent. *Journal of Indian Society of Soil Science,* 39: 89-93

Yadegari, M., Asadi R, H. and Noormohammadi, G. 2010. Plant growth promoting Rhizobacteria increase growth, yield and nitrogen fixation in *Phaseolus vulgaris*. *Journal of Plant Nutrition*, 33: 1733–1743

Yoshiba, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. 1997. Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiology*, 38: 1095–1102

Zahir, Z. A. and Arshad, M. 1996. Effectiveness of *Azotobacter* inoculation for improving potato yield under fertilized conditions. *Pakistan Journal of Agricultural Science*, 33: 1-5

Zahir, Z. A., Ghani, U., Naveed, M., Nadeem, S. M. and Asghar, H. N. 2009. Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase for improving growth and yield of wheat *Triticum aestivum* L. under salt-stressed conditions. *Archives of Microbiology*, 191:415–424

Z**ahir, Z. A., Arshad, M. and Frankenberger, W. T. Jr. 2003.** Plant growth promoting rhizobacteria: Applications and perspectives in agriculture. *Advances in Agronomy*, 81: 97–168

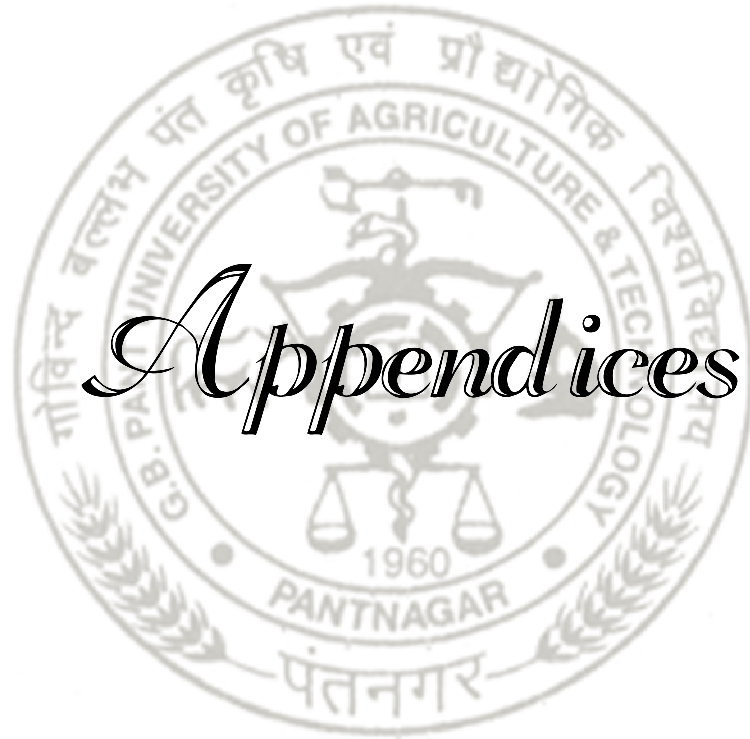
Zahir, Z. A., Munir, A., Asghar, H. N., Shaharoona, B. and Arshad, M. 2008. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas *Pisum sativum* under drought conditions. *Journal of Microbiology and Biotechnology*, 18: 958–963

Zahran, H. H. 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *Journal of Biotechnology*, 912(3):143-153

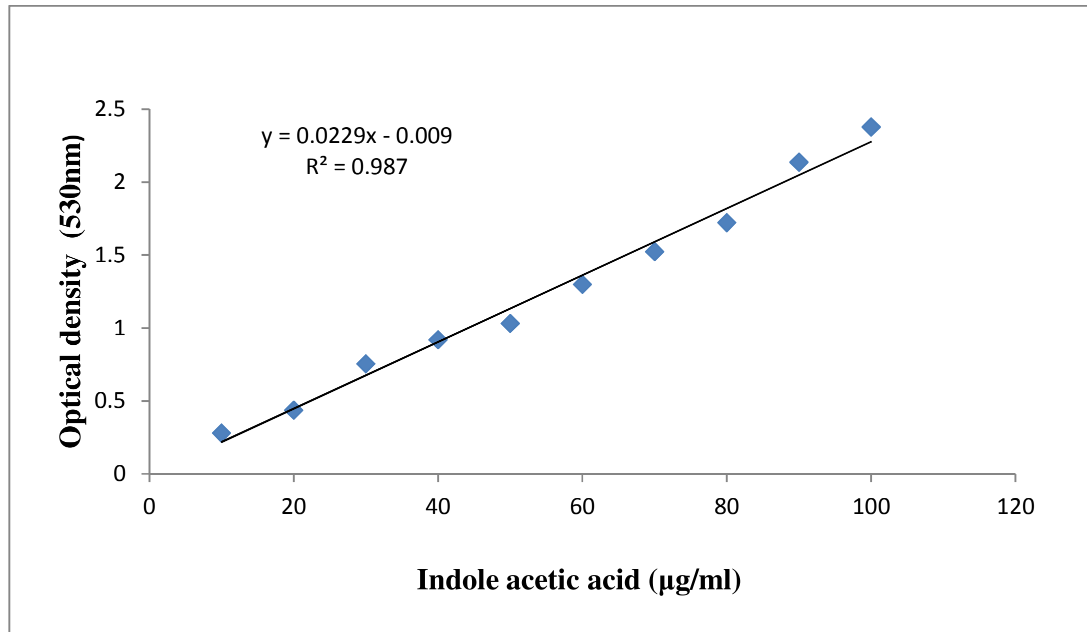
Zaidi, A., Khan, M. S., Ahemad, M. and Oves, M. 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiol Immunol Hung*, 56: 263–284

Zhong, Y. and Shangguan, Z. P. 2014. Water consumption characteristics and water use efficiency of winter wheat under long-term nitrogen fertilization regimes in northwest China. *PLoS One,* 9: 98850– 98857

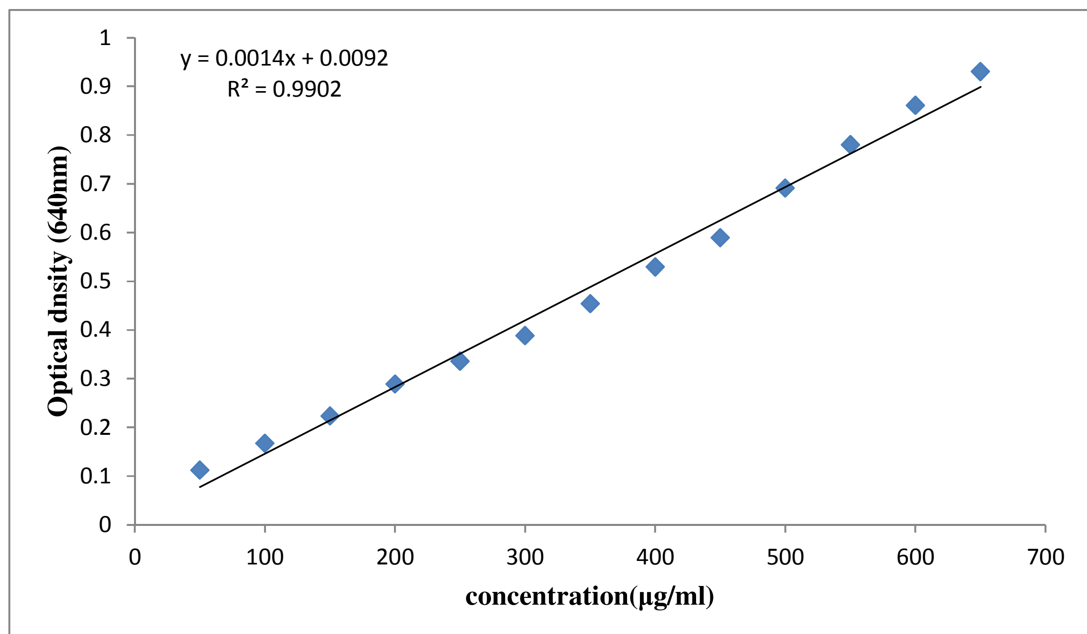
Appendices



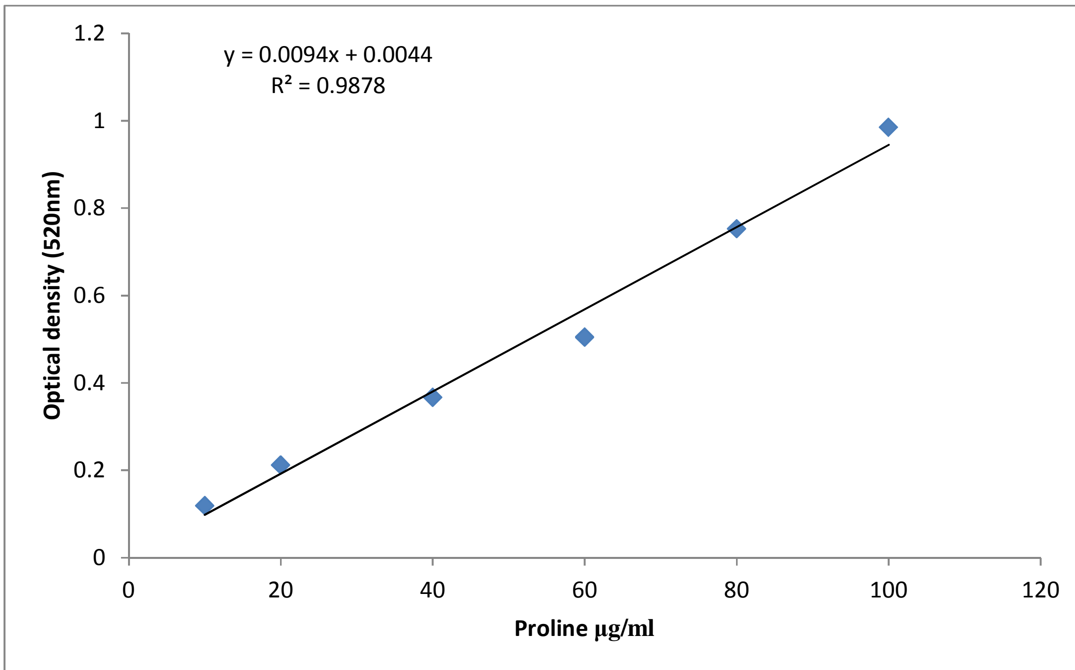
Appendix I



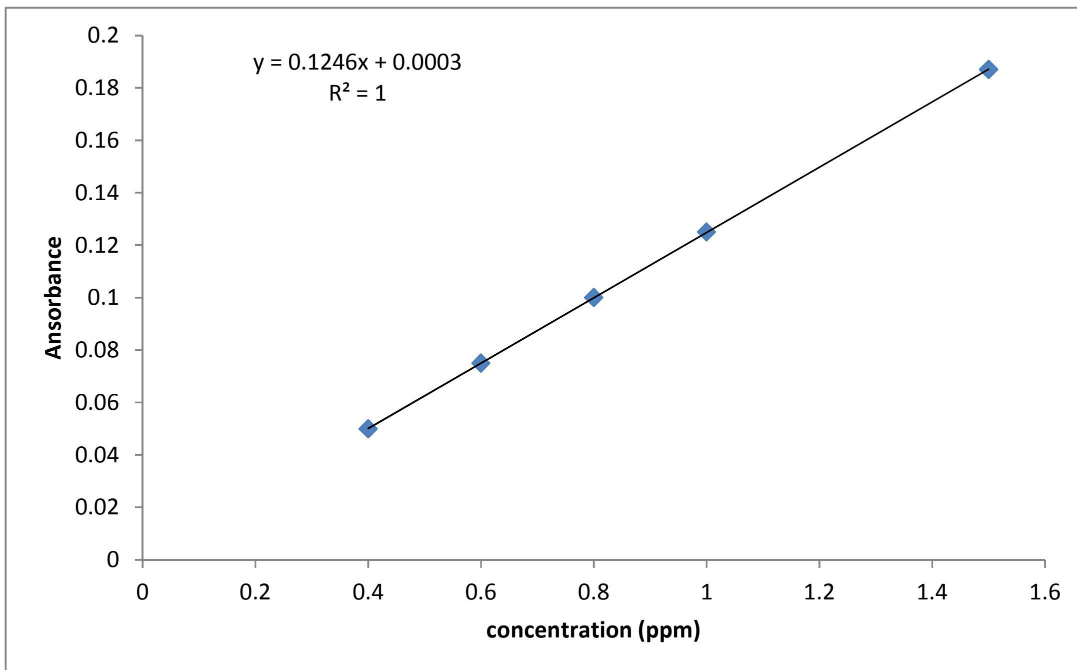
Standard curve of Indole acetic acid (IAA)



Standard curve of Phosphate solubilization



Standard curve of proline



Standard curve of zinc solubilization

Appendix II

1. **Nutrient agar**

	gl^{-1}
Beef extract	3.0
Peptone	5.0
Agar	20.0
pH	7.2±0.2

2. **Chrome azurol 'S' agar medium**

Dye solution

(A) Chrome-azurol 'S'	60.60mg
Distilled water	50 ml
(B) 1mM $FeCl_3.6H_2O$ in 10 mMHCl	10.0 ml
(C) Hexadecyltrimethyl ammonium bromide (HDTMA)	72.90mg
Distilled water	40 ml
Total volume of dye solution	100 ml

Solution **(A)** was added to solution **(B)** then this solution was added to solution **(C)**. The resultant dark blue solution is autoclaved and added to 300 ml autoclaved nutrient agar before pouring.

3. **Pikovaskya's agar**

	gl^{-1}
Yeast extract	0.5
Dextrose	10.0
Calcium phosphate	5.0
Potassium chloride	0.2
Magnesium sulphate	0.1
Manganese sulphate	0.0001
Ferrous sulphate	0.001
Agar	20.0
pH	7.0±0.2

4.	**Glucose yeast extract peptone agar**	**gl^{-1}**
	Glucose	40.0
	Peptone	5.0
	Yeast extract	5.0
	Agar	15.0

5.	**Skim milk agar**	**gl^{-1}**
	Skim milk powder	28.0
	Casein enzyme hydrolysate	5.0
	Yeast extract	2.5
	Dextrose	1.0
	Agar	15.0
	pH	7 ±0.2

6.	**EPS producing medium**	**gl^{-1}**
	Peptone	10.0
	Meat extract	3.0
	Sodium chloride	5.0
	Sucrose	2.0
	pH	6.5±0.2

7.	**CMC agar medium**	**gl^{-1}**
	KH_2PO_4	1.0
	$MgSO_4.7H_2O$	0.5
	NaCl	0.5
	$FeSO_4.7H_2O$	0.01
	$MnSO_4.H_2O$	0.01
	NH_4NO_3	0.3
	CMC	10.0
	Agar	12.0
	pH	7±0.2

At the end of the incubation, to visualize the hydrolysis zone, the agar medium is flooded with an aqueous solution of Congo red (1% w/v) for 15 min. The Congo red solution is then poured off, and the plates were further treated by flooding with 1 M NaCl for 15 min.

8.	**Christensen's urea agar**	**gl^{-1}**
	Peptone	1.0
	Dextrose	1.0
	NaCl	5.0
	Potasssium phosphate	2.0
	Urea	20.0
	Phenol red	0.012
	Agar	15.0
	pH	6.8±0.2

Directions: Suspend 24 grams in 950 ml distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 1150C for 20 minutes. Cool to 500C and aseptically add 50 ml of filter sterilzed urea solution and mix well.

9.	**Nutrient gelatin**	**gl^{-1}**
	Peptic digest of animal tissue	5.0
	Beef extract	3.0
	Gelatin	120.0
	pH	6.8±0.2

10.	**Pectin agar medium**	**gl^{-1}**
	Pectin	5.0
	Yeast extract	1.0
	Agar	15.0
	pH	5.5±0.5

11.	**Potato Dextrose agar**	**gl^{-1}**
	Potato infusion	200
	Dextrose	20.0
	Agar	20.0
	pH	5.6±0.2

12.	**Peptone water**	**gl^{-1}**
	Peptic digest of animal tissue	10.0
	Sodium chloride	5.0
	pH	7 ±0.2

13. Zn solubilizing (mineral salt media)

Ingredient	gl^{-1}
Glucose	10.00
$(NH_4)_2SO_4$	1.0
KCl	0.2
K_2HPO_4	0.1
$MgSO_4.7H_2O$	0.2
$ZnO/ZnCo_3$	0.1%
Agar	2.0
pH	6.0±0.2

14. NBRIP broth

Ingredient	gl^{-1}
Glucose	10.0
Tricalcium phosphate	5.0
Magnesium chloride hexahydrate	5.0
Magnesium sulphate heptahydrate	0.25
Potassium chloride	0.2
Ammonium sulphate	0.1

15. Glucose yeast extract peptone agar

Ingredient	gl^{-1}
Glucose	40.0
Peptone	5.0
Yeast extract	5.0
Agar	15.0

16. Tryptic soy agar medium

Ingredient	gl^{-1}
Tryptone	17.0
Peptone (Soybean digest)	3.0
Glucose	2.5
NaCl	5.0
KH_2PO_4	2.5
Agar	15.0
pH	7.0±0.2

17. Starch agar

Ingredient	gl^{-1}
Peptone	5.0
Beef extract	3.0
Soluble starch	10.0
Agar	15.0
pH	7.4±0.2

Appendix III

GRAM STAINING-

1. Crystal violet

Solution A

Crystal violet	2g
Ethyl alcohol (95%)	20ml

Solution B

Ammonium oxalate	0.8g
Distilled water	80ml

2. Gram's iodine

Iodine	1g
Potassium iodide	2g
Distilled water	300ml
Sodium bicarbonate	3g

3. Destaining solution

Ethyl alcohol	95ml
Gram's iodine/Distilled water	5ml

4. Safranin

Safranin (2.5% solution in 100ml ethyl alcohol)	10ml
Distilled water	90ml

Appendix IV

ENDOSPORE STAINING-

1. Malachite green

Malachite green	0.5g
Distilled water	100ml

2. Destaining solution

Ethyl alcohol	95ml
Gram's iodine/Distilled water	5ml

3. Safranin

Safranin (2.5% solution in 100ml ethyl alcohol)	10ml
Distilled water	90ml

VITA

The authoress of this manuscript, Ms. Manisha Panwar was born on 14Jan 1995 at Srinagar, Uttrakhand, India. She passed high school in 2011 from Kendriya Vidyalaya Upper Camp, Dehradun and higher secondary education from Kendriya Vidyalaya O.L.F. in 2013 with first division. She obtained graduate degree in Bachelor of Science in 2016 with first division. She was admitted to the College of Post Graduate Studies in Department of Microbiology, CBSH, in 2016in G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, for the degree of M.Sc. with major in Microbiology. During Post Graduation, she was also awarded with Graduate Teaching Assistantship of University.

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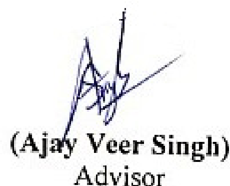
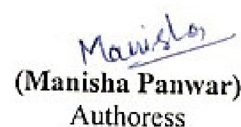
Thesis Title	: **"Microbial consortia development and their evaluation for the growth of wheat under drought stress conditions"**		
Advisor	: Dr. Ajay Veer Singh		

ABSTRACT

Drought considered as one of the major abiotic stress that severely affects overall plant growth and productivity worldwide compare to any other abiotic stress. Therefore, sustainable approaches are needed to assist plants growth under drought stress in order to mitigate crop losses. The present study was designed to develop microbial consortia of plant growth-promoting rhizobacteria and their bio-protective effects on wheat and determined shelf life of microbial consortia under different bioformulation preparations. In the current investigation nine bacterial isolates were selected as prominent drought tolerant bacteria on the basis of better growth potential in nutrient broth supplemented with 10% poly ethylene glycol (PEG). Drought tolerant isolates showed remarkable plant growth promoting properties such as production of siderophore, ACC deaminase, exopolysaccharide and efficient solubilization of zinc and phosphate and selected for consortium development on the basis of their biocompatibility assay. These bacterial consortia and their individual bacterial isolates were further tested comparatively for different plant growth promotory functional traits. The biochemical study of the bacterial isolates also confirmed their ability to produce various extracellular enzymes and utilization of diverse substrates as their carbon source. Furthermore, plant growth promotory potential of microbial consortia were assessed through *in situ* pot experiment in greenhouse with wheat (*Triticum aestivum* var. PBW 373) plants inoculated with or without consortia under drought stress conditions. The results of *in situ* pot experiment confirmed the efficiency of consortia to confer drought stress tolerance and retained higher biomass as compared to non-inoculated plants. Inoculated plants showed high content of chlorophyll and carotenoids, higher relative water content and proline content, significantly lower MDA content in the vegetal tissues hence confirmed the PGPR mediated amelioration of drought tolerance in wheat. Furthermore, bioformulation of bacterial consortia were prepared by using talc and sugarcane bagasse as carrier and evaluated for shelf life on different conditions for 90 days. The survival rate of bacterial consortia was higher in talc-based bioformulation at 90th day at 4 ºC. Above results demonstrated that these developed bacterial consortia could be used as bio-inoculant after further evaluation under field conditions to enhance crop productivity under drought stress for sustainable agriculture.



(Ajay Veer Singh)
Advisor



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Authoress

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सत्र एवं प्रवेष वर्ष	: प्रथम, 2016–2017	**उपाधि**	: स्नातकोत्तर
प्रमुख	: सूक्ष्मजीव विज्ञान	**विभाग**	: सूक्ष्मजीव विज्ञान

शोध का शीर्षक : **माइक्रोबियल कंसोर्शिया का विकास एवं इनका सूखे तनाव की स्थिति के तहत गेहूँ के विकास के लिए मूल्यांकन**

सलाहकार : डॉ. अजय वीर सिंह

सारांश

सूखा प्रमुख अजैविक तनावों में से एक हैं जो कि सम्पूर्ण पादप वृद्धि एवं उत्पादन को गंभीर रूप से प्रभावित करता है। अतः सूखा तनाव की स्थिति में पादप वृद्धि में सहायक एवं फसल की हानि को कम करने के लिए दीर्घकालिक प्रस्ताव की आवश्यकता है। वर्तमान अध्ययन की रूपरेखा, पादप वृद्धि में सहायक राइजोबैक्टीरिया के सूक्ष्म जीव समूहों के विकास एवं इनका गेहूँ पर जैव सुरक्षात्मक प्रभाव और विभिन्न जैवसूत्रीकरण के द्वारा सूक्ष्मजीव समूहों की जीवन अवधि निर्धारित करने के लिए बनाई गई हैं। वर्तमान जाँच में नौ पृथक जीवाणुओं को 10% पॉलीइथाइलीन ग्लाइकॉल अनुपूरित पोषक ब्रॉथ में बेहतर वृद्धि क्षमता के आधार पर सूखा सहनशील जीवाणुओं के रूप में चुना गया। सूखा सहनशील जीवाणुओं द्वारा उल्लेखनीय पादप वृद्धि गुण जैसे कि सिड्रोफोर,एसीसी डीएमिनेज,एक्सोपॉलीसैकेराइड उत्पादन एवं जस्ता व फॉस्फेट घुलनशीलता दर्शाई गई एवं जैव सुसंगति परीक्षण के आधार पर सूक्ष्मजीव समूहों का सृजन किया गया। इन जीवाणु समूहों औंर उनके एकल जीवाणुओं को विभिन्न पादप वृद्धि को बढ़ाने हेतु तुलनात्मक रूप से परीक्षित किया गया था। पृथक जीवाणुओं के जैव रासायनिक अध्ययन ने कोशिका अतिरिक्त एंजाइमों के उत्पादन तथा विभिन्न कार्बन स्रोतों के उपयोग की पुष्टि की। इसके अतिरिक्त जीवाणु समूहों की पादप वृद्धि क्षमता का आकलन गेहूँ (*ट्रिटिकम एस्टिवम* प्रजाति पी.बी.डब्लू.373) के साथ हरित गृह में *इन सीटू* पॉट परीक्षण के द्वारा जीवाणु समूहों के साथ एवं बिना जीवाणु समुहो के साथ सूखा तनाव की स्थिति में किया गया। *इन सीटू* पॉट परीक्षणों के परिणामों ने जीवाणु समुहों के सूखा तनाव सहनशीलता की पुष्टि की तथा गैर संचरित पौधों की तुलना में अधिक जैव भार धारण किया। संचरित पौधों के वनस्पतिक ऊतक में उच्च क्लोरोफिल एवं कैरोटिनॉइड़ मात्रा उच्च, सापेक्षिक जल मात्रा, प्रोलीन की मात्रा में अधिकता तथा एम.डी.ए मात्रा में सार्थक कमी दर्शाई। अतः पी. जी. पी. आर. की मध्यस्तथा से गेहूँ में सूखा सहन क्षमता में उन्नति की पुष्टि हुई। इसके अतिरिक्त टाल्क एवं गन्ने की खोई को वाहक के रुप में उपयोग करके जीवाणु समुहों का जैव सुत्रीकरण तैयार किया गया था तथा विभिन्न दशाओं में 90 दिनों तक इनके जीवनकाल का आलंकन किया गया। जीवाणु समूहों के जीवित रहने की दर टाल्क आधारित जैव सुत्रीकरण में 90 दिनों में 4 डिग्री सेल्सियस की स्थिति में सबसे अधिक थी। उपरोक्त परिणाम दर्शाते है कि विकसित किये गये जीवाणु समुहो का पुनः आलंकन करने के उपरांत खेत एवं सूखा तनाव की परिस्थितियों में वायोइनोकूलेन्ट की तरह कृषि उत्पादकता बढ़ाने हेतु टिकाऊ कृषि में प्रयुक्त किये जा सकते है।

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