पादप वृद्धि प्रोत्साहक राइज़ोबैक्टीरिया का गेहूं (*ट्रिटीकम एस्टिवम* एल.) की उत्पादकता एवं पोषकतत्व उपयोग दक्षता पर प्रभाव

Effect of Plant Growth Promoting Rhizobacteria on Productivity and Nutrient Use Efficiency of Wheat (*Triticum aestivum* L.)

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Effect of Plant Growth Promoting Rhizobacteria on Productivity and Nutrient Use Efficiency of Wheat (*Triticum aestivum* L.)

By

AMIR JAN Dawlatzai

A Thesis

Submitted to the Faculty of Post Graduate School,

Indian Agricultural Research Institute, New Delhi,

in partial fulfilment of the requirements

for the award of degree of

MASTER OF SCIENCE

In

AGRONOMY
2015

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Dedicated to my partents and wife for the endless love, support and ecouragment.

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This is to certify that the thesis entitled **"Effect of Plant Growth Promoting Rhizobacteria on Productivity and Nutrient Use Efficiency of Wheat (*Triticum aestivum* L.)"** submitted to the of Post-Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi, in partial fulfilment of requirement of degree **of Master of Science in Agronomy**, embodies the result of a *bona fide* research work carried out by **Mr. Amir Jan** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

All the assistance and help received during the course of investigation has been duly acknowledge.

Date: **7Th June, 2015**
Place: **New Delhi**

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ABBREVIATIONS

LSD	:	Least significant difference
SEm±	:	Standard error of mean
DAS	:	Days after sowing
NS	:	Non-Significant
HI	:	Harvest index
P or P_2O_5	:	Phosphorus
₹	:	Indian rupee
cm	:	Centimetre
K or K_2O	:	Potassium
et al.	:	And others
mg	:	Milligram
kg	:	Kilogram
Max.	:	Maximum
Mini.	:	Minimum
M	:	Meter (s)
N	:	Nitrogen
No.	:	Number
ha	:	Hectare
viz.	:	Namely
%	:	Percent
i.e.	:	That is
°C	:	Celsius
t	:	Tonne
Fig.	:	Figure
g	:	Gram
µ	:	Micro
hrs	:	Hours

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Chapter – 1

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the important and strategic cereal crop for the majority of world's population. It is the most important staple food of about 50 % of the world population (Rana *et al.*, 2012). Worldwide, wheat provides nearly 55% of carbohydrates and 20% of food calories. Both in term of production (12.05%) and area (12.5%), India ranks second in the world (www.agricoop.nic.in). It was grown on 29.4 million hectares with production of 92.3 million tonnes and productivity of 3.1 tonnes per hectare in India during 2012-13 (SIA, 2013).

Although, the agricultural productivity has drastically increased during the twentieth century by the use of the high inputs, high yielding varieties, inorganic fertilizers, herbicides, pesticides and mechanization, whereas world still faces the problem of uncertainty of food security. Most important issue is the predicted increase in the global population from 7.125 billion in 2013 to 9 billion people by 2050. The solicitude of feeding the additional population will be more problematic in developing countries where the population will increase from 5.6 billion in 2009, to 7.9 billion in 2050 (http://www.unfpa.org/public/). Presently, the world population could be fed by the current level of food production (Pretty, 2008). But it is still unlikely that current growth in agricultural productivity can go side by side with increasing population (Hazell and Wood, 2008). In addition, most of the developing countries face environmental problems that will hinder the development of agricultural system's ablility to meet the future food grain production. These problems include decrease in irrigation water availability, increase in desertification, and reduced cultivable land area, etc. Possibly these constraints could further be aggravated by the ensuing climatic changes (Cummings, 2009).

In order to produce enough food, agriculture had relied on the application of large quantity of inorganic N fertilizer to the soil, but their use efficiency still remain low, caused by losses through volatilization, denitrification, leaching and conversion into unavailable forms (Sturz *et al*., 2000). Intensive use of chemical fertilizers destroys the soil ecology, disturb the environmental balance, degrades soil fertility, contaminates ground water and consequently leads to harmful effects on human health (Ayala and Rao 2002; Joshi *et al.*, 2006). Therefore, in such a scenario, supplementing nutrients by biofertilizers can be an appropriate and environment friendly alternative for increasing crop yields and sustain the inherent soil fertility.

Biological fertilizers, also known as bio-fertilizers, are products carrying livings cells of different types of microorganisms which are able to transform alimentary important elements

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(N, P…) from unavailable to available form by biological means such as N fixation and solubilization of rock phosphate (Narula *et al.*, 2000; Sahu and Jana, 2000; Cakmakci *et al.*, 2001; Vessey, 2003). Plant growth promotion in crop plants mainly results from the improved nutrient uptake or hormonal stimulation (Dobbelaere *et al.*, 2003) and reduced disease incidence (Kloepper and Schroth,1978). Plant growth promoting rhizobacteria (PGPR) includes an ample variety of soil bacteria which, when grown in association with host plant, leads to stimulation of growth of their host because of increased mobility, uptake and enrichment of nutrients in plant (Lucas *et al.*, 2004; Cakmakci *et al.*, 2006). The use of PGPR is increasing in agriculture. PGPR are known to engage one or more direct and indirect mechanisms of action to improve plant growth and health, however, the main mode of action of many PGPR is by increasing the availability of nutrients for plants in the rhizosphere (Glick 1995). PGPR play important role in different species of crops including cereals (Karthikeyan *et al.*, 2007; Selvakumar *et al.*, 2008; Prasanna *et al.*, 2009; Manjunath *et al.*, 2010; Nain *et al.*, 2010), horticultural (Baset *et al.*, 2010) and other crops (Khalid *et al.*, 2004; Fischer *et al.*, 2007; Gholami *et al.*, 2009). However, the increase in crop growth has been generally evaluated in terms of crop yields.

One of the important mode of action of PGPR is to decrease the dependence on the application of chemical N_2 fertilizers by fixing atmospheric nitrogen through biological processes (Dobereiner, 1997; Rodriguez *et al.*, 1996). Symbiotic nitrogen fixators have generally been used in legume production and currently, there is a growing interest in the use of free nitrogen fixators in other agricultural production systems (Casanovas *et al.*, 2000). Asymbiotic N_2 fixing bacteria which live in the rhizosphere have been reported to increase yields of cereals and other crops (Reinhold and Hurek, 1989). The estimated supplementation of free-living N_2 fixing prokaryotes to the N input of the soil ranges from 0-60 kg ha^{-1} $year^{-1}$ (Bürgmann *et al.*, 2003).

Furthermore, PGPR can also synthesize phytohormones which are also known as plant hormones. These Phytohormones are auxins, cytokinins, gibberellins, abscisic acid, and ethylene (Zahir *et al.*, 2004). The production of auxins, cytokinins, gibberellins, and abscisic acid is well known characteristic of rhizobia (Phillips and Torrey, 1970). They play key role in physiological processes and development of plant (Chiwocha *et al.*, 2003). In addition, they also prevent and promote stem elongation, improvement of fruit color, and avoid leaf falling (Ijaz, 2009).

In addition, ethylene is known as a repining hormone. It improves adventitious root and root hairs development, encourage germination and break down the dormancy of the seed (Pratt

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and Goeschl, 1969). Moreover, presence of ethylene with high concentration after germination inhibits root elongation. Ethylene level is lowered in plants by synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzymes. It is a function of PGPR (Reid, 1987). Ethylene is changed to ammonia and α-ketobutyrate which they can be used by bacterium as source of nitrogen and carbon (Honma and Shimomura, 1978). This is how, the bacterium acts as a sink for ACC and decrease the level of ethylene in the plants. Plants which are inoculated with ACC-deaminase producing bacteria can have longer roots (Glick *et al*., 1999). PGPR inoculation has been found to increase root weight (Bashan and Dubrovsky, 1996). More importantly, increases in the root length and root surface area are sometimes reported (Galleguillos *et al.*, 2000). Indole-3-acetic acid, a phytohormone of auxin class, is known to be involved in root initiation, cell division and cell enlargement (Salisbury, 1994). This hormone is very commonly produced by PGPR (Barazani and Friedman, 1999). The reporting of root length and root surface area are important because increase in these parameters are more reflective of an increase the volume of soil explored, than that which would be indicated by just increases in root weight.

Considering all these points in view, a field experiment entitled **"Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)"** was conducted with the following objectives:

Objectives:

i. To find out the effect of plant growth promoting rhizobacteria (PGPR) on root and shoot growth, and productivity of wheat,
ii. To estimate the NPK uptake and grain quality of wheat under varied treatments of PGPR inoculation, and
iii. To work out the economics of PGPR inoculation in wheat

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Chapter – 2

REVIEW OF LITERATURE

The review of literature on important aspects pertaining to present study is being presented in this chapter. There have been extensive studies on the performance of the PGPR (plant growth promoting rhizobacteria) both in pot and field experiments. The studies have been carried out on the various species of the PGPR and their plant growth promoting characteristics has been widely published. In this chapter, research work conducted in India and abroad on the influence of the PGPR on various characteristics of wheat and other crops has been reviewed.

2.1. Plant growth promoting rhizobacteria (PGPR)

Despite the fact that bacteria were not known to exist until the discovery of microscopic animals by Anton von Leeuwenhoek (1683), their use to promote plant growth has been exploited since ancient times. Theophrastus (372–287 BC) suggested the mixing of various soil samples for correcting defects and adding heart to soil (Tisdale and Nelson, 1975). Virgil recorded the establishment of legumes on cultivated land and reported the beneficial effects of legume crops in increasing the fertility of soil (Chew, 2002). Hellriegel and Wilfarth (1888) studied the rhizosphere root colonization in grasses and legumes and proposed the ability of soil bacteria to convert atmospheric N_2 into plant usable forms. Kloepper and Schroth (1978), while carrying out an experiment on radishes, introduced the term 'rhizobacteria' to the soil bacterial community that competitively colonized plant roots and stimulated growth and therefore decreasing the occurrence of plant diseases. Kloepper and Schroth (1981) called these beneficial rhizobacteria as plant growth promoting rhizobacteria (PGPR). PGPR are defined as the indispensable part of rhizosphere biota, when grown in association with the host plants are able to promote growth of the host. PGPR are successful in getting established in soil ecosystem because of their high adaptability in a wide variety of environments, rapid growth rate and biochemical versatility to metabolize a wide range of natural and xenobiotic compounds. Cook (2002) treated PGPR as the important component in the management of agricultural practices with inherited genetic potential.

The term PGPR has now been confined to the bacterial strains that can fulfil at least two of the three criteria such as aggressive colonization, plant growth stimulation, and bio-control (Weller *et al.*, 2002; Vessey, 2003). According to Whipps (2001) there are three basic types of interactions (neutral, negative or positive) mainly happens between the rhizobacteria and

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growing plants. Most rhizobacteria associated with plants are commensals in which the bacteria establish harmless interaction with the host plants leaving no visible effect on the growth and overall physiology of the host (Beattie, 2006). In negative interactions, the phytopathogenic rhizobacteria produces phytotoxic materials such as hydrogen cyanide or ethylene, therefore, negatively affecting the growth and physiology of the plants. The third types of PGPR exert a positive effect on plant growth by the direct mechanisms such as solubilization of nutrients, nitrogen fixation, production of phytohormones, etc. or by the indirect mechanisms such as stimulation of mycorrhizae development, competitive exclusion of pathogens or removal of phytotoxic substances (Bashan and de-Bashan, 2010).

PGPR can also be classified according to their association with the plant root cells into two types, viz., extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martinez-Viveros *et al.*, 2010). The ePGPR may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex; whereas iPGPR colonize generally inside the specialized nodular structures of root cells. The bacterial genera like, *Agrobacterium, Arthrobacter, Azotobacter, Azospirillum, Bacillus, Burkholderia, Caulobacter, Chromobacterium, Erwinia, Flavobacterium, Micrococcous, Pseudomonas and Serratia* belong to ePGPR (Gray and Smith, 2005). The iPGPR contain the endophytes and *Frankia* species both of which are able to symbiotically fix atmospheric N_2 with the higher plants (Verma *et al*., 2010). Endophytes include a wide range of soil bacterial genera such as *Allorhizobium, Azorhizobium, Bradyrhizobium, Mesorhizobium* and *Rhizobium* of the family Rhizobiaceae that mainly occupy the root systems in crop plants to form nodules (Wang and Martinez-Romero, 2000) and enhance growth either directly or indirectly. This group of rhizobacteria is mainly Gram-negative and rod-shaped with a lower proportion being Gram-positive rods, cocci and pleomorphic. Examples for this group are *Allorhizobium undicola* (de Lajudie *et al*., 1998a), *Azorhizobium caulinodans* (Dreyfus *et al*., 1988), *Bradyrhizobium japonicum* (Guerinot and Chelm, 1984), *Mesorhizobium chacoense* (Velazquez *et al*., 2001), *Mesorhizobium pluriforium* (de Lajudie *et al*. 1998b), *Rhizobium ciceri* (Nour *et al*., 1994), *Rhizobium etli* (Segovia *et al.*, 1993), *Rhizobium fredii* (Scholla and Elkan, 1984), *Rhizobium galegae* (Lindstrom, 1989), *Rhizobium gallicum* (Amarger *et al*., 1997), *Rhizobium giardinii* (Amarger *et al*., 1997), *Sinorhizobium arboris* (Nick *et al*., 1999), *Sinorhizobium fredii* (Chen *et al*., 1988) and *Sinorhizobium medicae* (Rome *et al*., 1996).

In addition to the importance of PGPR in maintaining root health, nutrient acquisition, and tolerance to environmental stress (Malhotra and Srivastava, 2009), but the specific traits of

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plant growth promotion are limited at a given environment of plant–microbe interactions. Many PGPR formulations are now available as commercial products for agricultural production of most crops. In recent years, the use of PGPR to promote plant growth has increased in various parts of the world. De Datta (1981) suggested that soil microbial flora lead to a number of biochemical changes in the soil which highly affects soil fertility. For many years, organic farmers have been suggested that instead of applying nutrient to the plant, it is better to feed the soil and let the soil to feed the plant (Magdoff and Van, 2000). Studies in Madagascar have found that application of 1-2 t ha^{-1} of PGPR-enriched compost can have almost equal positive effect on yield over applying 4,6–8 t ha^{-1} compost without PGPR enrichment (Randriamiharisoa, 2001).

Soil microbial communities are important for maintaining biological balance in the soil, which play key role in the sustainability of either natural ecosystem or agro ecosystems (Kennedy and Smith, 1995). PGPR can enhance growth and yield of crop plants by direct and indirect mechanisms. In some PGPR species, plant growth promotion dominates with nitrogen fixation, phosphate solubilization and production of phytohormones, like auxin and cytokinin and volatile growth stimulants such as ethylene and 2, 3-butanediol (Ryu *et al*., 2003; Vessey, 2003). Siderophore production for rhizosphere colonization has also been reported as one of the important mechanisms by certain PGPR (*Bradyrhizobium japonicum, Rhizobium leguminosarum and Sinorhizobium meliloti*) (Carson *et al*., 2000; El-Tarabily and Sivasithamparam, 2006) with plant growth promoting activity. In addition to iron-chelating, siderophores (Schippers *et al*., 1988), antibiotics (Weller, 1988), and hydrogen cyanides (Stutz *et al*., 1986) may also to be made by PGPR strains, involved enormously in the reduction of phytopathogens and harmful rhizobacteria with a corresponding enhancement in plant health. Finally, regardless of beneficial effect on the plant growth, it is prerequisite for PGPR to colonize rhizosphere or root itself (Glick, 1995).

2.2. Plant-microbes interactions

Plant microbe interactions may happen at phyllosphere, endosphere and rhizosphere. Phyllosphere belongs to the aerial parts of the plants and endosphere related to the internal transport root system. But the term Rhizosphere, can be defined as any volume of soil, particularly influenced by the plant roots or it is in association with the roots and plant produced material. According to Bringhurst *et al.* (2001) rhizosphere, generally includes the region of soil bound by plant roots, usually extending a few mm from the root surface. Rhizosphere of

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soil is much richer in bacteria than the surrounding bulk soil (Hiltner, 1904). Reports based on molecular techniques have estimated more than 4,000 microbial species per gram of soil (Montesinos, 2003). Filamentous actinobacteria are also treated as one of the key community in rhizosphere microbiota (Benizri *et al*., 2001) and are capable of influencing the plant development as well as protect the plant roots against phytopathogens. Plant exudates like amino acids and sugars supply a rich source of energy and nutrients for the bacteria in rhizosphere, leading to more microbial populations in the region than outside the region (Haas and Defago, 2005).

Plant-root interactions in rhizosphere may involve root-root, root-insect, and root-microbe interactions, which leading to the production of more root exudates that eventually favors maximum microbial populations (rhizosphere engineering) in this ecologically important region. Modification in rhizobacterial community structure have been stated with the application of polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) leading to a significant change in plant–microbes interactions (Herschkovitz *et al*., 2005). Nevertheless, effective root colonization and persistence of PGPR in plant rhizosphere are necessary in order to exert their beneficial effect on the plant (Elliot and Lynch, 1984). The affection between the plants and the environment in rhizosphere is thus crucial for better uptake of water and nutrients by plants as well beneficial interactions of plants with soil-borne microorganisms (Ryan *et al*., 2009). According to Cardoso and Freitas (1992) the rhizosphere microbial communities are actively associated with the biogeochemical cycling of nutrients like C, P, N, and S, removal of toxins and production of phytohormones or antibiotics etc. Rhizobacteria may rely on other microbes for nutrient sources as one microbe may convert plant exudates into a form that can be used by another microbe. Therefore, rhizosphere has showed up as a versatile and dynamic ecological environment of intensive plant-microbe interactions (Mayak *et al*., 2004) harnessing essential micro and macro-nutrients affecting plant growth, even though, the process of root colonization is under the effect of different parameters such as bacterial traits, root exudates and many other biotic and abiotic factors (Benizri *et al*., 2001).

In many rhizospheric relationships, the PGPR are accepted to colonize the plant roots (Andrews and Harris, 2000) and stimulate plant growth. The colonization of plant rhizosphere by *Azospirillum* sp., *Bacillus subtilis* sp., and *Pseudomonas* sp., has been reported by Steenhoudt and Vanderleyden (2000), and Trivedi *et al*. (2005). In addition, immobilized form of PGPR inoculants over free forms has more ability of survival and plant root colonization.

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Recently, it has been stated that soil microorganisms, including free-living as well as associative and symbiotic rhizobacteria belonging to the genera such as *Acinetobacter, Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Proteus, Pseudomonas, Rhizobium, Serratia, Xanthomonas* in particular, are the integral parts of rhizosphere biota (Glick, 1995 and Kaymak, 2011) resulting to successful rhizosphere colonization.

Lugtenberg *et al*. (2001) showed that a large number of cell surface molecules are responsible for the effective rhizosphere colonization. Rhizospheric colonization is therefore, considered as a critical step in the application of microorganisms for beneficial purposes such as biofertilization, phytostimulation, biocontrol and phytoremediation, however, the colonization of rhizosphere by PGPR is not a uniform process. For example, *Kluyvera ascorbata* colonized the upper two-thirds of the surface of canola roots but no bacteria were observed around the root tips (Ma *et al.*, 2001).

2.3. Plant growth

Soil microorganisms live in the rhizosphere of the plants. They have very close relationship with plant roots but, their effect may be multifarious. There are several associated bacteria living in the rhizosphere, which have strong positive effect on the plants, particularly, the plant growth promoting rhizobacteria (PGPR). PGPR include beneficial bacteria strains that can grow in the rhizosphere and stimulate plant growth (Vessey, 2003; Yolcu *et al*., 2012). PGPR increase the plant growth through production of phytohormones, biological nitrogen fixation, and increased solubility of insoluble essential elements in soil (Rovera *et al*., 2008; Rosas *et al*., 2005).

Some studies concluded that the inoculation of wheat with PGPR enhanced its growth characteristics. The studied bacteria included *Azospirillum* (Bashan and Levanony, 1990), *Azotobacter* (Rai and Gaure, 1988), *Bacillus* (Freitas, 2000), *Pseudomonas* (Zaidi and Khan, 2005), *Clostridium* (Gasoni *et al*., 2001), and *Herbaspirillum* (Baldani *et al*., 2000). Use of PGPR along with phosphate solubilizing bacteria (PSB) increased plant growth (Rudresh *et al*., 2005; Zaidi *et al.*, 2003). Mirzaei *et al.*, (2010) reported that application of *Azotobacter* and *Azospirillum* bacteria at different levels of nitrogen on sunflower increased plant growth characteristics and decreased nitrogen fertilizer application by 50%.

In addition, Manjunath *et al.* (2010) showed that application of *Providencia* sp. (WRB4) had significantly impacted wheat shoot weight, succeeded by combined inoculation of

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Anabaena oscillariodes + *Providencia*. In addition, the values of wheat root weight were recorded higher for application of *Providencia* sp. (WRB4). Similarly, crop biomass and vigor index values of wheat were significantly higher for application of *Providencia* sp. whereas in term of root length application of *Providencia* sp. WRB4 and combined application of *Anabaena torulosa* and *Alcaligenes* gave higher values. Furthermore, Nain *et al*. (2010) concluded that plant parameters like, shoot weight, root weight and total biomass of wheat was observed highest with inoculation of *bacterial* strains (PW1 + PW5 + PW7) and *Cyanobacteria* (CW1+ CW2 + CW3).

There are many reports on the positive effects of the inoculation of wheat with *Azotobacter* or/and *Azospirillum* (Tawfik and Gomaa, 2005, Abbasdokht 2008, Badr *et al.*, 2009, Bahrani *et al*., 2010). Tilak (1992) showed positive effects of combined inoculation of *Azotobacter* and *Azospirillum* on dry matter production of maize and sorghum.

Egamberdiyeba (2007) reported that bacterial strains *Pseudomonas alcaligenes* PsP15, *Bacillus polymyxa* BC P 26 and *Mycobacterium phlei* M6P19 had a much stimulatory effect on plant growth and N, P and K uptake of maize in nutrient deficient calcisol soil. Their stimulatory efficiency decreased in nearly rich loamy sand soil where bacterial inoculants stimulated only root growth and N, K uptake of root. In addition, Khalid *et al.* (2004) showed that inoculation of wheat seedlings with PGPR under gnotobiotic (axenic) conditions increased root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 17.3%) and shoot dry weight (up to 36.3%) over control.

Steenhoudt and Vanderleyden (2000) reported that PGPR are capable of producing phytostimulators which directly increase plant growth. In addition to fixing atmospheric nitrogen, *Azospirillum* spp. are also able to give off phytohormones, such as, auxins, cytokinins and gibberellins. In addition, Das and Saha (2003) reported that inoculation of soil with *Azospirillum* in partial application of nitrogen fertilizer showed highest stimulation of these microaerophilic N_2-fixing bacteria in the rhizosphere.

2.4. Yield attributes

PGPR strains have been found to have a positive effect on different yield attributes of several crops. Abd EI- Lattief (2013) reported a significant increase in spike length, number of spikelets/spike, kernel weight /spike, 1000-kernel weight of wheat with inoculation of *Azotobacter* and *Azospirillum*. Further, Biswas *et al.* (2000a) showed that rice inoculation with strain E-11 or IRBG-74 of *Rhizobium leguminosarum* bv. trifolii increased number of panicles

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per plant and filled grains panicle^{-1}, and total number of spikelets plant^{-1} over uninoculated plants. In addition, Peng *et al.* (2006) also reported that *Rhizobial* inoculation increased sink size by increasing either the panicle number or spikelet number per panicle. They also showed that increased spikelet number per panicle was higher at 90 kg N ha^{-1} over zero N application. Furthermore, Choudhary *et al*. (2010) reported that inoculation of *Azospirillum brasilense* and *Bacillus subtilis* resulted in statistically similar number of filled grains panicle^{-1}, and both showed significantly higher number of filled grains panicle^{-1} over no inoculation.

2.5. Grain and biological yields

Many studies have suggested that PGPR increased yield of several crops including wheat. In fact, PGPR is known to stimulate plant growth and thereby increasing the number of tillers, straw weight, and 1000-grain weight. Among the PGPR, *Azotobacter* is the most widely studied, which has been reported to increase the growth and yield of the wheat when applied alone or in combination with other PGPR. Esmailpour *et al.* (2012) reported that inoculation with *Azotobacter* increased the grain yield of wheat by 15% and biological yield by 9% as compared to control. In addition, *Azotobacter* had synergistic impact on the plant height as well. The results showed that plant height increased by 15%. In addition, Abd EI- Lattief (2013) showed that application of 75% mineral nitrogen and *Azotobacter* increased grain yield of wheat by 12.5% over recommended mineral N application. They further stated that application of 75% mineral N and *Azotobacter* resulted in 42.2% harvest index. Similarly, Kızılkaya (2008) reported that all *Azotobacter* strains increased the grain and straw yields of wheat, whereas the maximum increase was obtained from non-indigenous *Azotobacter* strain *Beijerinck* 1901 by 97% in grain yield and 33% in straw yield in a pot experiment under greenhouse condition. They also reported that in contrast to the indigenous *Azotobacter* strains like, TK39, RI48, AND RK49 showed promising performance by 74, 70 and 84% in grain yield and 69, 65 and 92% in straw yield, respectively under field condition.

Yousefi and Barzegar (2013) reported that application of 100% chemical fertilizer with *Azotobacter* and *Pseudomonas* increased biological yield of wheat by 12.9% as compared to application of 100% chemical fertilizer alone. Kumar *et al.* (2000) reported that application of mutant strains of *A. chroococcum* increased the grain yield (12.6%) and straw yield (11.4%) of wheat over control and their survival (12–14%) was higher in the rhizosphere as compared to their parent soil isolate. In addition, Hussain (1979) reported that inoculation of *A. chroococcum* increased cereal yield by 15-20%. Further, higher biological yields were achieved in wheat and barley seeds inoculated with *Azotobacter* and *Azospirillum* (Ali *et al.*, 2005). In addition,

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Pandey (1998) reported an increase in maize yield by 1.15 fold after inoculation with *Azotobacter* over control. Yield increase by more than 20% have also been achieved in wheat inoculated with *Azotobacter* (Khavazi *et al*., 2005). Similarly, Hassanpur *et al.* (2012) reported that mycorrhizae fungi, *Azotobacter* and their combined inoculation significantly enhanced grain yields of wheat by (21.6%), (13.3%), and (17.5%) respectively, over the control.

Manjunath *et al.* (2010) showed that the grain weight were recorded higher with application of *Providencia* sp. (WRB4) in wheat. Furthermore, Turan *et al.* (2010) showed that combined PGPR inoculation with the strain of OSU-142 + M-13 + *Azospirillum* sp. 245 have significantly enhanced grain yield of wheat over full doses of nitrogen application. Similarly, Rai and Caur (1998) reported that combined inoculation of *Azotobacter* and *Azospirillum* had positive effects on grain yield, biological yield, and harvest index in various wheat genotypes.

Khalid *et al.* (2004) showed that peat based seed inoculation with selected PGPR isolates exhibited stimulatory effects on grain yield of tested wheat cultivar in pot (up to 14.7% enhancement over control) and field experiment (up to 27.5% increase over control); however, the response varied with the cultivar and PGPR strains. They concluded that the strain, which produced the highest amount of auxins in non-sterilized soil, also caused maximum increase in growth and yield of the wheat.

Thus it is evident from the above mentioned findings that inoculation of different crops, especially wheat, with PGPR had a positive effect on both the grain and straw yields. PGPR strains were found effective when inoculated singly or combined with other PGPR along with chemical fertilizers. In most cases the use of PGPR helped to save nitrogenous fertilizers. So, biofertilizers can substitute some portion of the chemical fertilizers besides having some other beneficial effects on the crop.

2.6. Root growth and morphology

Roots are an important part of the wheat morphology. They play a key role in giving anchorage to the plant and help the plant to absorb water and nutrients. Higher root surface area increases plant access to both water and nutrients from soil. Fallik *et al.* (1994) showed that inoculation of maize with *Azospirillum brasilence* increased the proliferation of root hair which could dramatically increase the root surface area. Further, Glick, (1995) reported that inoculation of different plant species with *Azospirillum* had increased the root respiration rates. Generally, IAA-producing PGPR are known to increase root growth and length of root, which will increase root surface area leading increased access of plants to nutrients from the soils. In

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addition, Kaci *et al*. (2005) isolated a strain of *Rhizobium* (KYGT207) from an arid soil in southern Algeria. They reported significant increase in shoot dry mass (85%), root dry mass (56%), root adhering soil (RAS) dry mass (dm) per root dm (RAS/RT) up to 137% and in RAS aggregate water stability by inoculation of wheat with the *Rhizobium* strain KYGT207.

Egamberdiyeva (2007) reported that the bacterial strains *Pseudomonas alcaligenes PsA15*, *Bacillus polymyxa BC P 26* and *Mycobacterium phlei* M6P19 had a much better stimulatory effect on plant growth, and N, P and K uptake of maize in nutrient deficient calcisol soil. Their stimulatory efficiency reduced in relatively rich loamy sand soil where bacterial inoculants stimulated only root growth and N, K uptake of root. A study was conducted by Rekha *et al*. (2006) to investigate the efficiency of microbial inoculants after encapsulating in alginate supplemented with humic acid on plant growth. Two promising plant growth promoting rhizobacteria such as *Pseudomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 were inoculated. Highest increase in root length was of lettuce obtained with CC-pg104 free-cell inoculated plants, followed by plants inoculated with encapsulated CC-pg104. Choudhary *et al*. (2010) reported higher values for root length and dry weight with the inoculation of *Azospirillum brasilense*, which were at par to *Bacillus subtilis* inoculation in rice. Roesti *et al.* (2006) reported that the percentage of root colonization by AMF was significantly higher in the treatments containing a mycorrhizal inoculum over the untreated control in rain-fed wheat field.

The foregoing paragraphs suggest that inoculation with some specific PGPR helps in improving root growth. The root growth improvement in terms of surface area, weight, length, and proliferation of root hair has been reported by research studies made on different cereal crops.

2.7. Effect on the grain quality

Inoculation with efficient strains of plant growth promoting rhizobacteria (PGPR) can potentially influence grain quality of crops. There are studies which report an increase in the protein content of plants with PGPR inoculation. Hasanpour *et al.* (2012) reported highest amount for protein percentage (12.4%) with combined inoculation of mycorrhizae and *Azotobacter* in wheat. Further, Roesti *et al.* (2006) reported that protein content in wheat grain was significantly higher in PGPR inoculated crop over the control plants and maximum values were achieved when PGPR were co-inoculated with the AMF in rain-fed wheat field.

2.8. Effect on the soil biological properties

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Soil microbial biomass and soil enzymes are known as important indicator of soil quality because, their relationship to the soil biology, simple to measure, rapid responses to changes in soil management and high sensitivity to changes, leading from management and environmental factors (Max *et al*., 2001, Jimenes *et al*., 2002). Reason for the use of microbial and biochemical properties of soil as soil quality indicators is their central role in cycling of C and N and their sensitivity to change (Nannipieri *et al*., 1990). However, it is now well established that a great number of soil microorganisms are able to produce plant growth regulating substances (phytohormones) but still little has been done to exploit the influence of microbially-produced phytohormones on plant growth and development, partially due to our knowledge is still incomplete, but even more so because the processes involved are so complex.

2.8.1. Soil dehydrogenase activity

Soil dehydrogenase activity determination in soils was first introduced by Lenhard in year 1956. Afterwards, it has been largely used due to its simplicity over other quantitative methods. The method was more recently modified by Casida *et al*. (1964) and Von *et al*. (1991). It has also been found that measurement of changes in soil enzyme activities may provide a useful index of changes in soil quality measurement. The soil dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in soil. The basic idea of using soil enzymes activity as a measure of microbial indicators for soil fertility was introduced and established by Waksman (Waksman, 1992). Measurement of dehydrogenase activity represents immediate metabolic activities of soil microorganism at the time of the test.

Soil dehydrogenase activity is an oxidative degradation process .i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrate to acceptors. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters. Manjunath *et al.* (2010) reported that highest dehydrogenize activities were reported with the application of *Providencia* sp. (WRB4) with 2/3 application of recommended N.

2.8.2.Soil fluorescein diacetate (FDA) hydrolysis

Hydrolysis of fluorescein diacetate, a colourless compound, to fluorescein which is coloured (Adam and Duncan, 2001), is also assessment of the contribution of several enzymes, viz. non-specific esterases, proteases and lipases, all of which take part in the decomposition of

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organic matter in soil. Since more than 90% of the energy flow in a soil system passes through microbial decomposers and heterotrophic microorganisms are predominantly in soil, FDA hydrolysis is thought to reflect overall soil microbiological activity. Nain Lata *et al*. (2010) observed highest values for FDA in soil with the application of two bacterial strains (PW_1, PW_7) and one cyanobacteria strain (CW_2).

2.8.3.Soil microbial biomass carbon (SMBC)

The soil microbial biomass is generally a living part of the soil organic matter. It is involved significantly in nutrient transformation, xenobiotic degradation, as a source and sink of C, N, P, S, and enhancing physiochemical properties of the soil (Angers *et al.*, 1992; Gupta and Germida, 1988). Because of its dynamic character, it has been shown to be a sensitive indicator of differences in soil quality under sustainable cropping systems (Anderson and Domsch, 1989; Karlen *et. al.*, 1997). It has also been used to compare microbial carbon and nitrogen content and nutrient cycling between soils under different management systems (Franzluebbers *et.al.* 1995; Doran and Smith, 1987; Carter and Rannie, 1982).

2.9. Nitrogen fixation

Nitrogen (N) is the most important nutrient for plant growth and productivity. However, 78% of nitrogen existing in the atmosphere is unavailable for the growing plants. The atmospheric N_2 is converted into plant available forms by biological nitrogen fixation (BNF). The BNF changes nitrogen to ammonia with help of nitrogen fixing microorganisms by using enzyme system known as nitrogenase (Kim and Rees, 1994). Indeed, BNF accounts for about two-thirds of the nitrogen fixed globally, while the remaining nitrogen is industrially synthesized by the Haber–Bosch process (Rubio and Ludden, 2008). Biological nitrogen fixation generally happens in mild temperatures, by nitrogen fixing microorganisms, which they are widely distributed in nature (Raymond *et al*., 2004). In addition, BNF serve as an economically beneficial and environmentally friendly alternative to chemical fertilizers (Ladha *et al*., 1997).

Nitrogen fixing organisms are mainly classified as (a) symbiotic N_2 fixing bacteria including members of the family rhizobiaceae. This family forms symbiotic relationship with leguminous plants (e.g. *Rhizobia*) (Ahemad and Khan, 2012; Zahran, 2001) and non-leguminous trees (e.g. *Frankia*), and (b) non-symbiotic (free living, associative and endophytes) nitrogen fixing includes such as *Cyanobacteria* (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*,

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Gluconoacetobacter Diazotrophicus and *Azocarus* etc. (Bhattacharyya and Jha, 2012). Although, non-symbiotic nitrogen fixing bacteria generally provide a small quantity of the fixed nitrogen which bacterially-associated host plant requires (Glick, 2012). Symbiotic nitrogen fixing rhizobia within the rhizobiaceae family (α-proteobacteria) infect and create symbiotic relationship with the roots of leguminous plants. The formation of the symbiotic relationship involves a complex interplay between host and symbiont (Giordano and Hirsch, 2004) leading to the formation of the nodules wherein the rhizobia colonize as intracellular symbionts. On the other hand, plant growth-promoting rhizobacteria which fixes N_2 in non-leguminous plants are also called as diazotrophs. They are able to form a non-obligate relationship with the host plants (Glick *et al.*, 1999). Plant growth promoting rhizobacteria not only have the ability to fix atmospheric N_2 in rang of 20-30 kg ha^{-1} (Table 2.1) (Prasanna *et al*., 2014) but also produce plant growth hormones similar to gibberellic acid and indole acetic acid, which could enhance plant growth, absorption of nutrients, and photosynthesis (Fayez *et al*., 1985).

Table 2.1 Nitrogen saving through use of biofertilizers

Types of biofertilizers	Crops	Amount of nitrogen mobilized ha^{-1}	Other benefits
Rhizobium	Legumes/pulses/oilseeds	20-30 Kg N	PGP and improved seed quality
Azotobacter	Cereal and fodder crops, vegetables, oilseeds	15-20 Kg N	PGP and improved seed quality
Azospirillum	Several grasses/cereals, fodder crops and non-leguminous crops	20-30 Kg N	PGP, especially root-system
BGA/ *Cyanobacteria*	Mainly in rice, but suitable for wheat, vegetables	25-30 Kg N	PGP, improved soil aggregation
PGP-Plant-growth promotion, AM- Arbuscular-mycorrhizae, BGA- Blue green algae, VAM-vesicular-arbuscular mycorrhizae			

(Prasanna *et al*., 2014)

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The process of N_2 fixation is performed by a complex enzyme, known as nitrogenase complex (Kim and Rees, 1994). Structure of nitrogenase was described by Dean and Jacobson (1992) as a two-part metalloenzyme consisting of (i) dinitrogenase reductase which is the iron protein and (ii) dinitrogenase which has a metal cofactor. Dinitrogenase reductase provides electrons with high reducing power while dinitrogenase uses these electrons to reduce N_2 to NH_3. Based on the metal cofactor three different N_2 fixing systems have been identified (a) Mo-nitrogenase, (b) V-nitrogenase and (c) Fe-nitrogenase. Structurally, N_2-fixing system varies among different bacterial genera. Most biological nitrogen fixation is carried out by the activity of the molybdenum nitrogenase, which is found in all diazotrophs (Bishop and Jorerger, 1990).

2.10. Increased phosphorus availability

Phosphorus (P) was first discovered by Brandt in 1669. The word phosphorus is derived from Greek, "phos" meaning light and "phorus" meaning bringing. It is the tenth most plentiful nutrient and it constitutes around 0.12% of the earth crust (Van Wazer, 1958). Soils generally contain 0.013-0.155% P and the insoluble phosphate compounds constitute 95-99% of the total P (Hayman, 1975). It is unevenly distributed along the soil profile and the usual content of the total P in the top soil is 500-800 mg/kg (Cole and Stevenson, 1999) which is equivalent to 1,100-1,800 kg P/ha in the plough layer. In Indian soils, the total P content ranges from 120 mg/kg in the arid ecological region of Rajasthan to 2,166 mg/kg in sub-humid temperate highlands of Himachal Pradesh (Blaise *et al.*, 2014). However, small portion of total P is available for the plant because of fixation in aluminium, calcium, iron, and magnesium and soil colloids. Therefore, the phosphate fertilizer efficiency is very low, especially in calcareous (Khalaffallah *et al.* 1982) and acid soil (Premono 1996).

Theoretical calculation have suggested that the accumulated P in agricultural soils is sufficient to sustain maximum crop yields worldwide for about 100 years (Goldstein *et al.*, 1993). The different methods to solubilize insoluble phosphate and enhance its availability by use of phosphate solubilizing microorganisms have been discussed by Illmer and Schimmer (1995). The role of microorganisms in increasing phosphate availability is by production of organic acids (Darmwal *et al.*, 1989), H_2S (Gaur, 1990), mineral acids (Kapoor *et al.*, 1991) and to H^+ protonation (Illmer und Schimmer, 1995) and mobilization of 20-30 kg P_2O_5 (Table 2.2) (Prasanna *et al*., 2014). Organic acids produced by microorganisms create stable complexes with phosphorus adsorbents (aluminium, iron and calcium) that is how enhances phosphate solubilization.

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On the other hand, organic P can constitute between 30-50% of the total P of the soil, a major proportion of it is corresponding to phytate (Borie *et al*., 1989; Turner *et al*., 2003). In this context, there are bacteria which can produce phytase enzymes for the mineralization of phytates (Lim *et al*., 2007; Jorquera *et al*., 2008). Much research work have shown that *Azotobacter chroococcum* enhances the growth of plants by different mechanisms, like nitrogen fixation (Lakshminarayana *et al*., 1992), ammonia excretion (Narula *et al*., 1980), phytohormones production (Azcon and Barea 1976), exudation of antifungal substances (Lakshminarayana *et al*., 1992), siderophore (Suneja *et al*., 1994) and phosphate solubilization (Kundu and Gaur 1980). In addition, Amer *et al.* (2002) reported that inoculation of wheat varieties with Phosphate solubilizing and phytohormones produced by *A. chroococcum* showed higher response over the control.

Table 2.2 Nutrient saving through use of biofertilizers

Types of biofertilizers	Crops	Amount of nutrient mobilized ha^{-1}	Other benefits
PSB (P solubilizers)	All crops	20-30 Kg P_2O_5	Plant-growth promotion
Mycorrhizae (AM/VAM)	Nursery crops, ornamentals, fruit orchards	20-30 Kg P_2O_5 + micronutrients	PGP and moisture conservations
AM- Arbuscular-mycorrhizae, VAM-vesicular-arbuscular mycorrhizae			

(Prasanna *et al*., 2014)

Arbuscular mycorrhizal fungi (AMF) can easily absorb mineral nutrients (George *et al*., 1995) through their lengthened hyphae networks, particularly from nutrient-poor soil and carry them to a host plant in exchange for carbohydrates. There are several reports that AM are able to produce organic acid (Lapeyrie, 1988; Paul and Sundara Rao, 1971) that could solubilize the insoluble mineral phosphates. The findings suggest that there could be further effects on the availability of Fe phosphates (Bolan *et al*., 1987; Cress *et al*., 1984).

Ectomycorrhizal fungi have generally been shown to possess P solubilizing activity (Lapeyrie *et al*., 1991). They are able to utilize P from inositol phosphates and possess phosphatase activity that could further affect their ability to release P from soil organic matter

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(Antibus *et al*., 1991; Koide and Schreiner, 1992). However, the use of AM as phosphate biofertilizers is limited by the inability to culture them in vitro, because they are obligate symbionts. Furthermore, AM infection is also dependent on the P status of the plant (Abbott *et al*., 1984). It is known that the AM fungi are not capable of colonizing plant roots strongly under P sufficient conditions (Koide and Schreiner, 1992) but is some specific cases the growth rates of plants were decreased by AM colonization in the presence of available P (Son and Smith, 1995).

Thus it is clear from the previous paragraphs that different PGPR play an important role in increasing the availability of phosphorus in soil. There are several mechanisms by which PGPR increase phosphorus availability in soil. The AM fungi solubilize and mobilize unavailable phosphorus into the plant roots.

2.11. Nutrient uptake

There are sufficient evidences that the mode of action of many PGPR is by increasing the availability of nutrients for the plant in the rhizosphere (Glick, 1995; Rodriguez and Fraga, 1999). The solubilization of P in the rhizosphere is the most accepted mode of action implemented in PGPR that enhances nutrient availability to host plants (Richardson, 2001). Examples of lately studied associations include *Azotobacter chrooroccum* in wheat (Kumar and Narula, 1999), *Bacillus circulans* and *Cladosporium herbarum* in wheat (Singh and Kapoor, 1999). Rhizobial inoculants may also encourage an increased number of roots hair and laterals, therefore facilitate higher nutrient uptake by exploration of a greater soil volume. Because certain strains of rhizobia are capable of solubilize precipitated P compounds (Chabot *et al*. 1996) and produce high affinity Fe-chelating siderophores (Guerinot, 1991). The likely contribution of these activities in enhancing the availability of rhizosphere P and Fe for uptake by plant roots needs to be explored.

Several plants exude or deposit a considerable part of their photosynthates produced in the canopy into the rhizosphere, 30-60% according to Pinton *et al*. (2000). Of this, 20-40% is exhausted into the root zone as carbon and other substrates for use by microorganisms. One of the nutritional benefits that aerobic microbes can provide to plants is solubilization of P. When P is analyzed in soil, this is generally reported as ‘available P’ only 10% of total P in the soil. Current studies in the UK found that the levels of soluble P in water runoff were increased 185-1,900% by altering wet and dry soil conditions (Turner and Haygarth, 2001). It was determined that aerobic bacteria were receiving P ions for their own growth from reserves in the soil that

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are unavailable to plants directly. When the soil was flooded, osmotic pressure used these bacteria so that their contents become available in the soil solution. When soil dried again, surviving aerobes resumed their growth and acquisition of 'unavailable' P. As this cycle of drying and wetting continued, more P was made 'available'. This suggests that the amount of P in the soil changing according to microbial activity.

Biswas *et al*. (2000b) showed that seed and seedlings of rice 'Pankaj' were inoculated with different *Rhizobia* and grown in potted soil supplemented with varied amounts of mineral N. Inoculation with *Rhizobium leguminosarum* bv. *trifolii* E11, *Rhizobium* sp. IRBG 74 and *Bradirhizobium* sp. IRBG 271, increased N, P and K uptake by 10-28% as compared to uninoculated rice plant. Furthermore, Habte and Osorio (2002) showed Mycorrhizal fungi absorb not only N, P and K, but also Ca, S, Fe, Mn, Cu and Zn from the soil and translocate them to plants in whose roots the fungi have established themselves. Lastly, Mycorrhizae increase the variety, as well as the quantity of nutrients becoming available to plants. In addition, they can stimulate hormone production in plants, enhance soil structure, suppress plant diseases, increase leaf chlorophyll levels, and enable plants to tolerate various kinds of stresses (Habte and Osorio, 2002).

2.12. PGPR as multifunctional inoculants

The effect of PGPR in crop productivity is not consistent under laboratory, greenhouse, and field trials. Because, soil is a changeable environment and a predicted result is sometimes difficult to obtain. Climatic variations also have a large effect on the capability of PGPR whereas sometimes unfavorable growth conditions in the field are to be expected as normal functioning of agriculture (Zaidi *et al.*, 2009). Plant growth promoting traits generally do not work independently of each other but additively as it was suggested in the ''additive hypothesis,'' that multiple mechanisms, such as phosphate solubilization, nitrogen fixation, ACC deaminase and antifungal activity, IAA and siderophore biosynthesis etc. are accountable for the plant growth promotion and increased yield (Bashan and Holguin, 1997). Under both natural agro-ecological niches and controlled soil environments, significant increase in yields of various crop plants has been obtained with applications of PGPR. Because of the existing reluctance worldwide to embrace foods produced by genetically modified plants, PGPR may be advantageous as a means of promoting plant growth. The wide scale application of PGPR may decrease the global dependence on agricultural chemicals.

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2.13. Rhizoremediation and stress control

Many reports on potential PGPR that degrade soil pollutants have been published worldwide. The contribution of the rhizomicrobial population to degrading pollutants allows plants to grow as natural vegetation at a contaminated site. Studies focused on degradation of compounds such as herbicides, pesticides and hazardous organic compounds have been carried out, however those reports have produced little information on the microbial population. An important step during rhizoremediation consist of the selection of pollutant degrading rhizobacteria that live in the rhizosphere and use the root exudates as an energy source (Kuiper *et al*., 2001). These bacteria, besides degrading the pollutant compounds, often directly assist rhizoremediation by producing hormones, fixing atmospheric nitrogen, solubilizing P or secreting siderophores (Denton, 2007). In the same way, consortia of bacteria are found to be efficient since each partner can accomplish different parts of the catabolic degradation route (Rahman *et al*., 2002).

When plants are exposed to stress conditions they respond increasing ethylene levels that lead to an increase in cell and plant damage (Argueso *et al*., 2007). A high concentration of ethylene can be harmful to the crop plants, because it induces defoliation and other cellular processes that may affect crop development (Desbrosses *et al*., 2009). Many PGPR damage 1-aminocyclopropane-1-carboxylate (ACC), a forerunner of the ethylene, through production of the enzyme ACC deaminase, which it leads to improve plant growth and development by decreasing plant ethylene levels. Furthermore, many forms of stress are relieved by ACC deaminase producers, like effects on phytopathogenic bacteria, and resistance to stress from polyaromatic hydrocarbons, and from salt and draught (Glick *et al*., 2007).

2.14. Phytostimulation

Diverse PGPR are able to alter root architecture and promote plant development due to their ability to synthesize and secrete plant hormones like indole-3-acetic acid (IAA), gibberellins (GAs), cytokinins and certain volatiles, therefore they are termed phytostimulators (Bloemberg and Lugtenberg, 2001). This capacity being bacterial strain specific (Boiero *et al*., 2007). The stimulatory effect of PGPR come from a manipulation of the complex and balanced network of plant hormones that directly are involved in growth or stimulation of the root formation. For example, the biosynthesis of IAA by different PGPR has been indicated to enhance root growth (Dobbelaere *et al*., 1999; Khalid *et al*., 2004). Bacteria use IAA to interact with plants as part of their colonization strategy, including phytostimulation and avoidance of basal plant defense mechanisms.

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It has recently been reported that IAA can also be a bacterial signaling molecule and therefore can have a direct effect on bacterial physiology (Spaepen *et al*., 2007). In bacteria there is no known role for GAs, rather they seem to be secondary metabolites that may play a role as signaling factors towards the host plant. In this way, there are many studies where gibberellins (GA) production by *Azospirillum* or *Bacillus* sp. induces growth promotion in plants (Bottini *et al*., 2004; Piccoli *et al*., 1997; Gutiérrez-Manero *et al*., 2001). Involvement of PGPR cytokinins were noticed in root initiation, cell division, cell enlargement and increase in root surface area of crop plants via improved formation of lateral and adventitious roots (Salamone *et al*., 2005; Werner *et al*., 2003). Some strains of *Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans, Rhodospirillum rubrum, Pseudomonas fluorescens, Bacillus subtilis,* and *Paenibacillus polymyxa* are reported to produce cytokinins (Glick 2012; Salamone *et al*., 2001). However, a comprehensive understanding of the role of PGPR synthesized cytokinins and how their production is regulated is not currently available.

It has recently been indicated that some rhizobacteria promote plant growth by releasing volatile signals (Ping and Boland, 2004). The discovery of *rhizobacterial* produced volatile organic compounds (VOCs), like 2, 3-butanediol, acetoin, terpenes, jasmonates, etc., constitutes an important mechanism for the elicitation of plant growth by rhizobacteria. The synthesis of bioactive VOCs seems to be a strain-specific phenomenon. The VOCs produced by the PGPR can act as signaling molecule to mediate plant microbe interactions as volatiles produced by PGPR colonizing roots are generated at sufficient concentrations to trigger the plant responses (Ryu *et al*., 2003). Still, more studies into the volatile components in plant rhizobacteria system should be carried out.

2.15. Resistance to drought

Drought stress generally causes limitation to the plant growth and productivity of agricultural crops especially in arid and semi-arid areas. Inoculation of plants with PGPR can increase the drought tolerance of the crops (Figueiredo *et al*., 2008) that might be because of the production of IAA, cytokinins, antioxidants and ACC deaminase. Inoculation of seeds of *Phragmites australis* with *Pseudomonas asplenii* improved germination and protect the plants from growth inhibition (Bashan *et al*., 2008). PGPR are also reported as beneficial to the plants such as tomatoes and peppers growing on water deficit soils for conferring resistance to water stress conditions (Aroca and Ruiz-Lozano, 2009).

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Diaz-Zorita and Fernandez-Canigia (2008) reported that a liquid formulation containing *Azospirillum brasilense* INTA Az-39 strain inoculated on wheat revealed more vigorous vegetative growth, with both greater shoot and root dry matter accumulation (12.9 and 22.0%, respectively). In addition, inoculation increased the number of harvested grains by 6.1%, and grain yield by 260 kg ha^{-1} (8.0%). More studies into the mechanisms by which PGPR elicit tolerance to a particular stress factors would increase our knowledge on the use of these rhizobacteria in agriculture to provide induced systemic tolerance to water stress under moisture stress conditions.

2.16. Influence of plant genotypes on PGPR functional groups

Plants at species, sub-species, and variety levels show considerable genetic and phenotypic diversity (Salamini *et al*., 2002; Vaughan *et al*., 2008). In the rhizosphere, different plant genotypes will have different influence on the number, diversity, and activity of microorganisms (Bais *et al*., 2006, Micallef *et al*., 2009). It was reported when comparing various plant species (Grayston *et al*., 1998, Costa *et al*., 2006, Berg and Smalla, 2009) or varieties within species (Germida and Siciliano, 2001; van Overbeek and van Elsas, 2008; Bouffaud *et al*., 2012). It entails differences clearly in root system structure, root exudation profile, and nutrient uptake (Czarnota *et al*., 2003; Comas and Eissenstat, 2009). These effects have also been proved where PGPR predominate.

Nitrogen fixing bacteria are specifically important for plant nitrogen nutrition (Hsu and Buckley, 2009; Turk *et al*., 2011). The analysis of functional groups showed that the size and/or composition of nitrogen fixing bacteria is affected by host plant features, both at plant species (Perin *et al*., 2006) and variety levels (Coelho *et al*., 2009; Wu *et al*., 2009). Analysis of *nifH* gene transcripts extracted from the rhizosphere indicated that only a fraction of the community expresses *nifH*, and that the corresponding bacterial species differed according to the plant variety, pointing to an influence of plant genotype on the functioning of nitrogen fixing bacteria (Knauth *et al*., 2005; Mårtensson *et al*., 2009; Orr *et al*., 2011). Same reports were presented with the functional group of phosphate solubilizers (Richardson and Simpson, 2011). Their selection by roots differs according to host plant species (Kaeppler *et al*., 2000; Chen *et al*., 2002; Ramaekers *et al*., 2010).

Thus it can be concluded that selective species of bacteria can enhance plant growth. These species live in, on or near roots and make the group, i.e. plant growth promoting rhizobacteria (PGPR). Some fungal species (e.g. AM-fungi) are also known to enhance the

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plant growth. The top most advantage of PGPR for crop plants would be atmospheric nitrogen fixation and solubilization of insoluble phosphorus. Hence, the availability of nitrogen and phosphorus is enhanced in the soil. The other important influence of PGPR on plant growth has been through the increased root growth and its activity. The PGPR inoculation causes enhanced nutrient uptake via improved root growth and nutrient availability. Such plants yield more dry matter and subsequently higher economic yield. Further, PGPR help the crop plants in resisting the adverse soil and environmental stresses.

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MATERIALS AND METHODS

The present experiment entitled **"Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)"** was conducted under the field condition during the winter (*rabi*) season of 2014-15. All the details including weather conditions, materials, and methods employed during the course of the investigation are presented in this chapter.

3.1 Details of experimental site

The experiment was conducted in the field (Main Block 14 C) at the Research farm of ICAR-Indian Agricultural Research Institute (IARI), New Delhi, located at a latitude of 28°40' N and longitude of 77°12' E, and an altitude of 228.6 meters above the mean sea level (Arabian Sea).

3.2 Climate and weather conditions

The climate of Delhi is of sub-tropical and semi-arid type with hot and dry summers and cold winters and falls under the agro-climatic zone 'Trans-Gangetic plains'. During summer, May and June months are the hottest with maximum temperature ranging between 41 and 46°C, while there is a decline in temperature from September onwards. January is the coldest month of the year with a minimum temperature ranging from 5 to 7°C. The mean annual rainfall is 650 mm, and July and August are the wettest months. The annual mean pan evaporation is about 850 mm. The detailed weather data during crop growing season recorded at the meteorological observatory of ICAR-Indian Agricultural Research Institute, New Delhi are given in annexure I and depicted in Figure 3.1. However, the lowest minimum temperature during the crop season was observed in the last week of December 2014 while the lowest maximum temperature occurred in the second week of January 2015 and the highest maximum temperature was observed during 3rd week of April 2015. A total of 315 mm rainfall was received during the cropping season, while the highest rainfall received in the second week of March, 2015.

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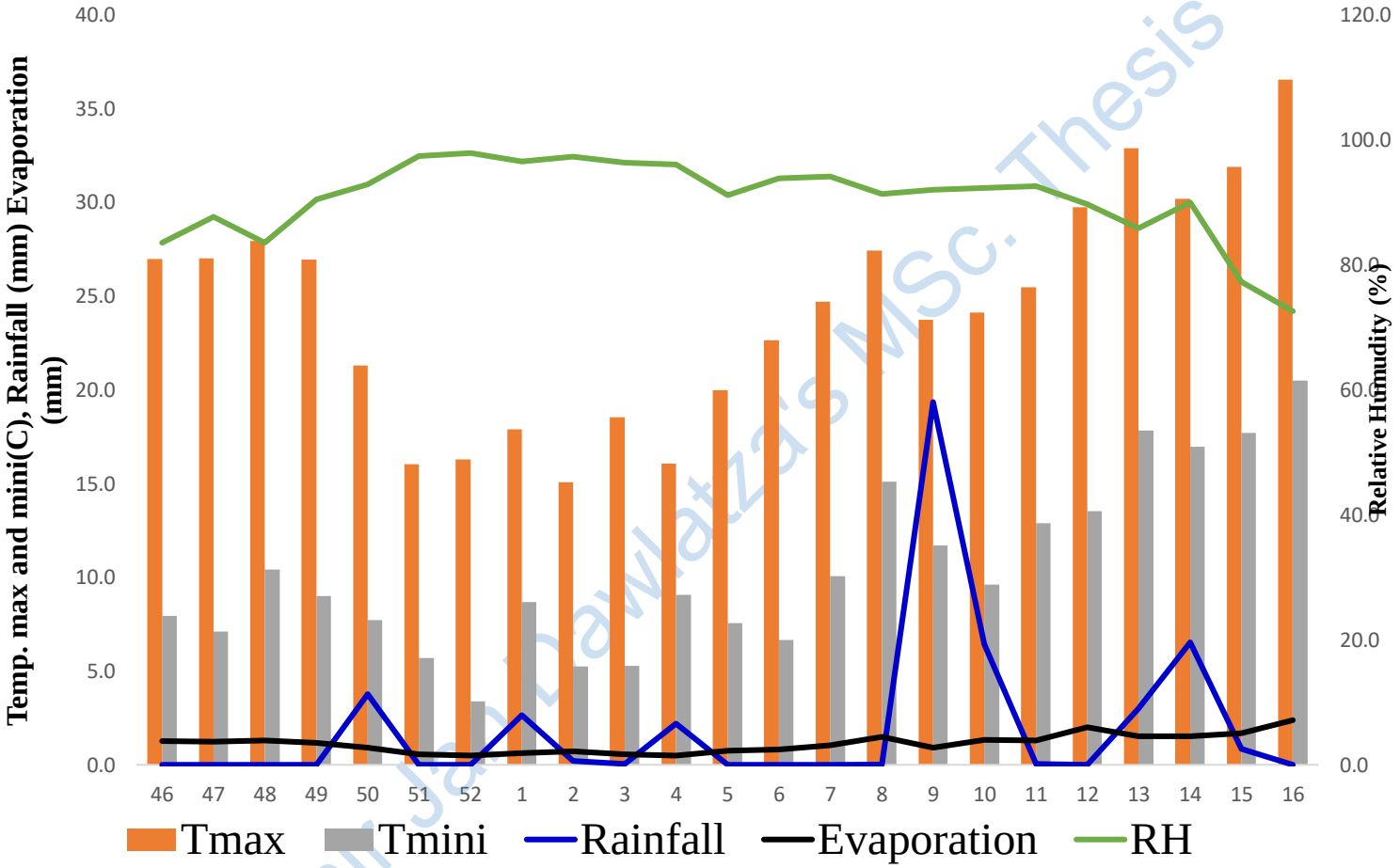

Fig. 3.1 Mean weakly weather parameter from Nov 2014-April 2015

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3.3 Soil characteristics

The soil of experimental site belongs to order Inceptisols, Mehruli series having sandy clay loam texture in upper 30 cm soil layer and loam below it. A composite representative soil sample was collected in 0-15 cm depth from the experimental field prior to start of the experimentation and analyzed for physico-chemical properties (Table 3.1). The soil was sandy clay loam in texture, poor in available N and medium in available P and organic carbon.

Table 3.1 Initial soil fertility status of the experimental field

S. N.	Particular	Content
A	**Physical characteristics**	
	Sand (%)	51.6
	Silt (%)	22.1
	Clay (%)	26.3
	Textural class	Sandy clay loam
	Moisture at 1/3 atmospheric tension (%) (Pressure plate apparatus, Richards and Weaver, 1943)	24.41
	Moisture at 15 atmospheric tension (%) (Pressure plate apparatus, Richards and Weaver, 1943)	11.2
	Bulk density (0-15 cm layer) (g cc^{-1})	1.48
B	**Chemical characteristics**	
	Organic Carbon (%) (Walkley and Black, 1934)	0.52
	Available N (N kg ha^{-1}) (Subbiah and Asija, 1956)	170
	Available Phosphorus (P kg ha^{-1}) (Olsen *et al*., 1954)	13.4
	Available Potassium (K kg ha^{-1}) (Flame photometer method, Hanway and Heidel, 1952)	260
	EC	0.82
	Soil pH (Cyber scam 500 pH meter)	8.2

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3.4 Cropping history of the experimental field

Since last five years the rice-wheat cropping system was continuously being adopted in the experimental area.

3.5 Experimental details

Crop: Wheat

Design: RBD

Replications: Three

Variety: HD–2967

Seed Rate: 100 kg/ha

Row Spacing: 22.5 cm

Gross Plot Size: 2.5m X 8.0 m

Total No. of Plots: 33

Water Management: As per the crop requirement

Treatments details:

T1 Absolute Control

T2 Control + *Azotobacter* (IARI Inoculant) + CW1 (*Anabaena sp*.) + PW5 (*Providencia sp*.)

T3 Recommended dose of NPK (RDF)

T4 75% N + Full dose PK

T5 75% N + Full dose PK + *Azotobacter* (IARI Inoculant)

T6 75% N + Full dose PK + CW1 (*Anabaena* sp.)

T7 75% N + Full dose PK + PW5 (*Providencia* sp.)

T8 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) + CW1 (*Anabaena* sp.)

T9 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) + PW5 (*Providencia sp*.)

T10 75% N + Full dose PK + CW1 (*Anabaena* sp.) + PW5 (*Providencia* sp.)

T11 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) CW1 (*Anabaena* sp.) + PW5 (*Providencia* sp.)

3.6 **Methodology**

Wheat crop was sown by Pora method with the help of hand plough at 22.5 cm rows distance during winter (*rabi*) season of 2014-15. Recommended dose of P (60 kg ha^{-1} P_2O_5 and K (40 kg ha^{-1} K_2O) was basal applied, as per treatment. The recommended dose of nitrogen (150 kg N ha^{-1}) was applied based on the treatment

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combination in two equal splits i.e. 50% basal and 50% second irrigation. Irrigation water was applied based on the crop requirement at the critical stages of the crop.

The crop received total number of 5 irrigations, plus one pre sowing irrigation. The first irrigation was applied before crown root initiation due to lower emergence of the crop and also to protect the emerged seedlings from birds attack. In addition, proper gap filling was carried out at 16 days after sowing (DAS) in plots where seed failed to emerge to maintain optimum plant population.

Weeds were controlled manually (hoeing) at the critical stage of the crop weed competition. Thus two hand weeding/ hoeing were carried out during the crop growth period at 17 DAS and 35 DAS. Herbicide application was avoided due to its deleterious effect on the soil microorganisms.

3.7 **Important characteristics of the wheat variety sown**

Wheat variety HD-2967 was used in the present study. It was developed by the Division of Genetics, ICAR-IARI, New Delhi. It was notified on 20th Oct, 2011. Its parentage is ALD/COC/URESH/HD 2160M/HD 2278. The variety is recommended for the cultivation in irrigated condition in the states of Punjab, Haryana, Delhi, Rajasthan, Uttar Pradesh, Jammu & Kashmir, Himachal Pradesh, and Uttarakhand under timely sown conditions.

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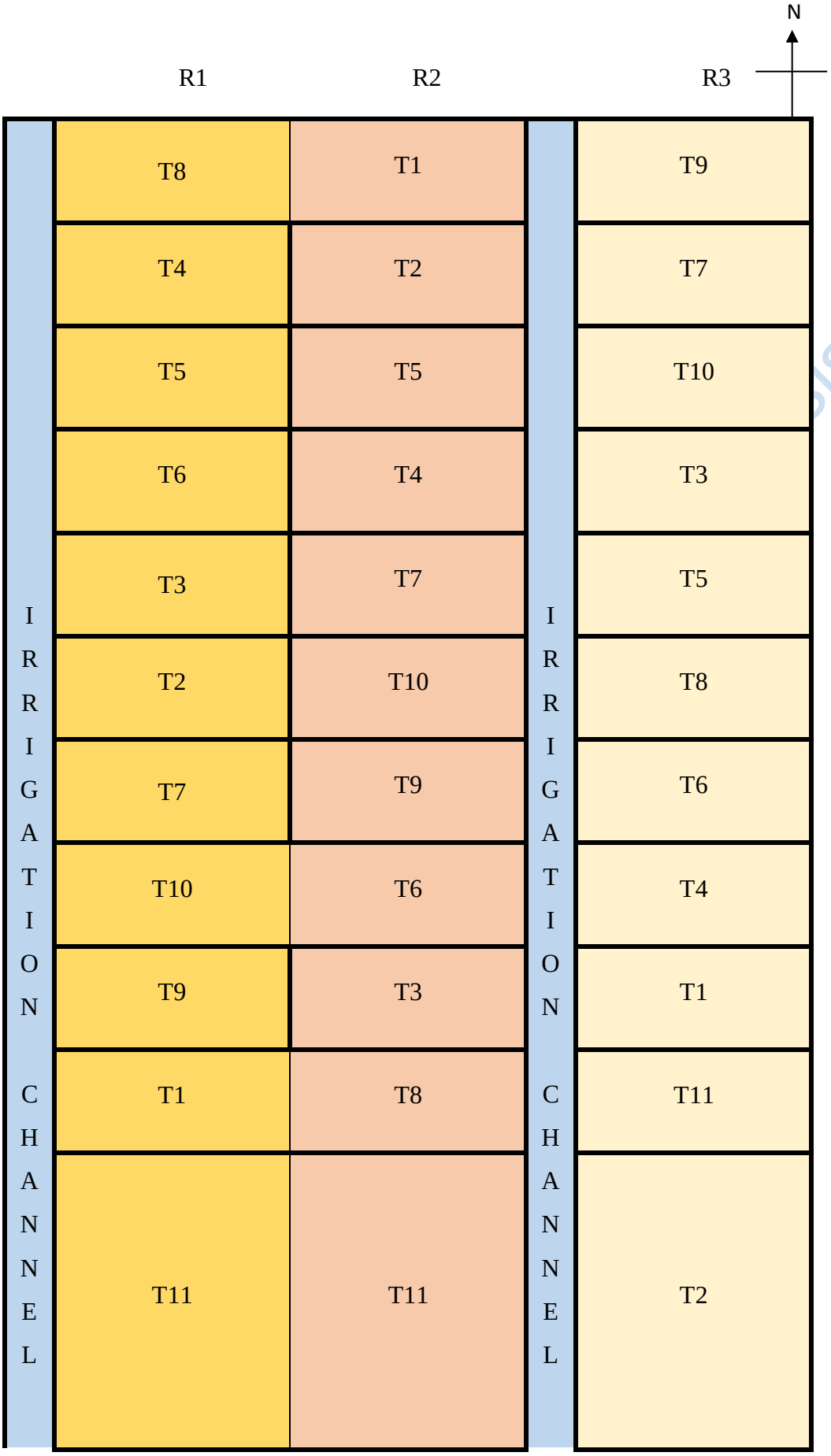

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Figure 3.2 Layout plan of the experimental field

3.8 Inoculation of PGPR

The bacterial strain *Providencia* sp. PW5 and cyanobacterial strain *Anabaena laxa* CW1 were obtained from the germplasm of the Division of Microbiology, ICAR-IARI, New Delhi. Their plant growth promoting activities had been earlier evaluated under gnotobiotic, net house and field conditions for rice and wheat crops (Nain *et al*. 2010; Prasanna *et al*. 2013; Rana *et al*. 2011, 2012). The log phase broth based inoculum of bacterial strain was prepared in nutrient broth under shaking conditions (150 rpm), at 28 ± 2ºC. The cyanobacterial strains were grown in BG 11 medium and incubated under optimal conditions of light and temperature (27 ± 2ºC and a light intensity of 52 – 55 µmol photon $m^{-2}s^{-1}$ and 16 L : 8 D Light: Dark cycles) for 14 d. The bacterial flasks were incubated in shaking incubator at 30 °C and 120 rpm for 48 hrs.

The culture suspensions were mixed with carrier (vermiculite: compost; 1:1) and used as described earlier with CFU (Colony Forming Units) maintained at 10^7-10^{10} g^{-1} of the bacteria and chlorophyll *a* content of 100 µg chlorophyll g^{-1} carrier (Manjunath *et al*. 2012; Prasanna *et al*., 2013). The *Azotobacter* inoculant was also obtained from the Division of Microbiology, ICAR-IARI, New Delhi. The formulations were amended with 1% CMC (carboxymethyl cellulose, from Himedia, India) as a sticker, prior to application on the seeds. The coated seeds were air dried in shade before sowing. All the formulations were used at the rate of 300 g per acre.

3.9 Field operations

Details of the field operation which were carried out during the experiment are presented in the Table 3.2.

3.10 Observation recorded

Methods which are followed for observation recording during the experimental period are presented below.

3.10.1 Growth parameters

3.10.1.1 Plant height

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Ten plants were randomly selected from a marked row in each plot and plant height was measured from the base of the plant at ground surface to the tip of the tallest leaf. The average value of plant height of ten plants was then computed and reported in centimeter (cm).

Table 3.2 Details of field operations

S. No.	Operation	Dates
1	Pre sowing irrigation	09 Nov 2014
2	Initial soil sampling	16 Nov 2014
3	Land preparation of experimental field	17 Nov 2014
4	Field layout	18 Nov 2014
5	Basal fertilizer application	18 Nov 2014
6	Sowing	18 Nov 2014
7	First irrigation	27 Nov 2014
8	Gap filling	03 Dec 2014
9	First hand weeding	04 Nov 2014
10	Second hand weeding	22 Nov 2014
11	Second irrigation	27 Nov 2014
12	Second split of N application	07 Jan 2015
13	Third Irrigation	01 Feb 2015
14	Fourth irrigation	22 Feb 2015
15	Fifth irrigation	02 April 2015
16	Harvesting and drying	17-20 April
17	Threshing, cleaning, drying and weighing	21-22 April

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3.10.1.2 Number of tillers

Numbers of tillers were recorded by counting the total numbers of tillers from the marked 0.5 m row length with the width of 0.225 m fourth row. Afterwards, it was converted to per m^2.

3.10.1.3 Dry mater accumulation

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Plants of 50 cm row length were harvested at different growth stages from the second and third rows. Samples were sun dried for 2-3 days and then oven-dried at 60±2°C for 24 hours and then dry weight was computed and has been reported in grams (g) m^{-2} at 30, 60, 90, 120 DAS and at harvest.

3.10.1.4 Leaf area index (LAI)

Plant samples (shoots) which were harvested for recording of dry matter production were also used to compute leaf area. The leaves were separated from the culm and cleaned with de-ionized water and then dried with tissue paper. The area of fresh green leaves for each treatment right after harvesting was measured by using leaf area meter (Model Number(s): LI-3100C). Leaf area index (LAI) was computed at 30, 60, and 90 DAS stage using the formula as described by Evans (1972). Leaf area index is expressed as the ratio of leaf surface (one side only) to the ground area occupied by the plant.

$$\text{LAI} = \text{Total leaf area } (cm^2)/\text{Ground area } (cm^2)$$

3.10.1.5 Mean crop growth rate

The dry matter data recorded at 30, 60, 90, 120 DAS and at harvest were used to for the computation of the CGR. It was expressed as g m^{-2} land area d^{-1}.The crop growth rate was calculated with the following formula (Watson *et al.*, 1952):

$$\text{CGR} = \left(\frac{W_2 - W_1}{T_2 - T_1}\right)\left(\frac{1}{S}\right)$$

Where,

W_1 and W_2 are dry weights (g) of plants at time T_1 and T_2, respectively

T_2- T_1 is the interval of time in days

S is land area (m^2) occupied by plants

3.10.1.6 Mean relative growth rate

The dry matter data recorded at 30, 60, 90, 120 DAS and at harvest were also used in RGR computation. It was expressed as mg g^{-1} dry matter d^{-1}. The relative growth rate was computed with the following formula (Watson *et al.*, 1952):

$$\text{RGR} = \frac{\text{Ln}W_2 - \text{Ln}W_1}{T_2 - T_1}$$

Where,

W_1 and W_2 are dry weights (g) of plants at time T_1 and T_2, respectively

T_2- T_1 is the interval of time in days

Ln is natural logarithm

3.10.1.7 Mean net assimilation rate

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The dry matter data recorded at 30, 60, 90, 120 DAS and at harvest were also used to compute the NAR. It was expressed as g m^{-2} leaf area d^{-1}. The net assimilation rate was worked out with the following formula (Watson, 1958):

$$NAR = \left(\frac{W_2 - W_1}{LA_2 - LA_1}\right)\left(\frac{LnLA_2 - LnLA_1}{T_2 - T_1}\right)$$

Where,

W_1 and W_2 are the dry weights (g) of plants at time T_1 and T_2 respectively

Ln is natural logarithm

T_2- T_1 is the interval of time in days

A_1 and A_2 are the leaf area (m^2) occupied by plants at time T_1 and T_2, respectively

3.10.1.8 Rood studies

For studying root length, volume, and dry weight of the crop, root samples were obtained from each plot from 0-15 cm depth with the help of a core soil sampler at flowering stage. The roots were cleaned by washing them carefully in the running water in a sieve. From the fresh roots, volume and length was measured by the help of a root scanner (Epson Expression 1640XL) and then root samples were dried in oven at 60^{o}C for 24 hours and weighed for recording root dry weight.

3.10.2 Yield attributes

3.10.2.1 Number of effective tillers

At harvest the total numbers of spikes from 1 m marked row length were counted from the each plot and then converted to m^{-2}.

3.10.2.2 Spike length

Length of ten randomly selected spikes from each plot was measured. It was measured from neck to the tip of the spike and then the average length was computed and reported in centimeter (cm).

3.10.2.3 Spike weight

The ten selected spikes used for spike length measurement, were also used to record the weight of the spike and mean spikes weight (g) was then computed and reported.

3.10.2.4 Number of filled grains

The total number of filled grains from the ten randomly selected spikes per plot were counted and their average was computed.

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3.10.2.5 Grain weight per spike

The ten selected spikes which were used for spike length measurement, were also used to record the grain weight of the spike and mean grain weight (g) was then calculated.

3.10.2.6 1,000-grain weight (g)

The 1,000-filled grains obtained from sampled spikes were first counted manually and then weighed to compute the 1,000-grain weight (g).

3.11 Yields and harvest index

3.11.1 Biological, grain and straw yields

The net plot area (separating 4 border rows on each side and 0.5 m from other sides of the Width) were harvested and then sun-dried for two days in the field and later the total biomass (biological) yield was recorded. After threshing, cleaning and drying the grain yield was recorded and reported at 14% moisture content. Straw yield was obtained by subtracting grain yield from the total biomass yield. The biological, grain and straw yields were expressed in t ha^{-1}.

3.11.2 Harvest index

The harvest index (%) was computed by using the formula given by Singh and Stoskofif, 1971.

$$\text{Harvest index (\%)} = \frac{\text{Economic yield (kg)}}{\text{Biological yield (kg)}} \times 100 = \frac{\text{Grain yield}}{\text{Grain yield + Straw yield}} \times 100$$

2.17. Soil biochemical parameters

Soil samples were collected from 0-15 cm soil depth from each plot. Soil samples were analyzed for microbial biomass carbon, FDA hydrolysis, dehydrogenase activity and soil chlorophyll. The collected soil samples were kept in a deep freezer until the analysis of the parameters was performed. The details of procedures for biochemical analyses are given below.

2.17.1. Microbial biomass carbon (MBC)

Microbial biomass carbon in soil samples was estimated by the method described by Nunan *et al.* (1998). The necessary reagents and estimation method follows.

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Reagent

1. Chloroform
2. 0.5M K_2SO_4: Prepared by adding 87.135 g of K_2SO_4 in one litre distilled water.

Estimation method

17.5 g soil sample was taken in a closed-capped bottle and then 1.0 ml chloroform was added to fumigate these samples. Simultaneously one non fumigated soil sample was set in a 250 ml flask. Then these samples were incubated in dark for 24 hours. After 24 hours of incubation, the chloroform was evaporated at 50°C in BOD i.e. opened the caps for next 20-24 hours. After that 70 ml 0.5M K_2SO_4 was added to samples and put on shaker for 30 minutes. Supernatant was taken out by filtering the samples with Whatman No. 42 filter paper. Absorbance of supernatant was recorded immediately for both fumigated and non-fumigated samples at 280 nM. Soil microbial biomass carbon (MBC) was computed by using the formula given below:

$$\mu g \text{ MBC/g soil (or mg of MBC/kg of soil)} = \frac{\text{O.D. with fumigated sample} - \text{O.D. with unfumigated sample}}{17.5} \times 15487$$

2.17.2. Fluorescein diacetate (FDA) hydrolysis

Microbial activity in terms of fluoresein diacetate (FDA) hydrolysis in soil was measured by procedure described by Green *et al*. (2006). The necessary reagents and estimation method follows.

Reagents

1. Fluorescein diacetate (FDA) stock solution: FDA (Hi Media) 10 mg was dissolved in 5 ml reagent grade acetone and stored in freezer.
2. 60 mM phosphte buffer (pH 7.6) : Take 0.7 g K_2MPO_4 in 400 ml distilled water (DW) + 1.3 g KH_2PO_4 in 400 ml DW and make up the final volume to one litre after adjusting the required pH (7.6).

Estimation method:

One gram soil sample was taken in a test tube containing 5 ml of 60 mM potassium phosphate buffer (7.6 pH) and 10 µl FDA stock solution and then

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incubated at 37°C at shaker for two hours. One control test tube for each sample prepared without adding FDA solution. Reaction was terminated by adding 0.2 ml (5% v/v) acetone reagent. Then after filterate it through Whatman no. 2 filter paper and absorbance of samples was recorded at 490 nM. FDA hydrolysis was computed in terms of A490 units" μg of Fluorescein released gram^{-1} soil h^{-1}"

2.17.3. Dehydrogenase activity

Dehydrogenase activity of soil samples was estimated by the method described by Casida *et al*. (1964). The necessary reagents and estimation method follows.

Reagents

1. Triphenyl tetrazolium chloride (TTC) : TTC (3.0 g) dissolved in 100 ml distilled water and stored in an amber coloured bottle at 4°C.
2. Methanol (AR grade)
3. Standard triphenyl formazan (100 μg ml^{-1}): 10 mg triphenyl (TPF) dissolved in 100 ml distilled water.

Estimation method

6 g fresh air-dried soil sample was saturated with 1.0 ml freshly prepared TTC (3% w/v) solution in a screw capped test tube then added pinch (0.1 g) of $CaCO_3$. Care was taken that no air bubble remains during packing of soil sample, rotated gently by shaking. These test tubes were incubated at 28±1°C (28-30°C) for 24 hours. After 24 hours TPF extracted (pink layer). Methanol (10 ml) was added to these test tubes and rotated it well for 1 min/sample. Supernatant was taken out carefully after allowing to stand for 10 minutes. Absorbance of supernatant was recorded by Spectrophotometer at 485 nm. Dehydrogenase activity was calculated and expressed in terms of μg TPF liberated g^{-1} soil h^{-1} or μg TPF g^{-1} soil day^{-1}.

2.17.4.Soil chlorophyll

Soil chlorophyll was analyzed using pre weighed soil cores (from 0-15 cm depth); acetone: DMSO (1:1) was added at the rate of 4 ml g^{-1}. The content were well shaken and they were incubated for 48-96 hrs. in the dark at room temperature. Intermittent shaking every 24 hrs. extracted the chlorophyll completely. Optical density were recorded at 663, 645, 630 and 775 nm and the chlorophyll a concentration determined (Nayak *et al*., 2004)

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Soil Chlorophyll (mg/g) = 11.64 (OD 663) – 2.16 (OD 645) + 0.10 (OD 630)

3.13 Soil chemical analysis

Soil samples were collected from 3 spots from each plot in 0-15 cm depth from the ground surface using soil auger. Soil samples were then air dried in the shade. The composite soil sample were analyzed for pH, organic carbon, Available N, available P, and available K. Available soil nitrogen was estimated by alkaline permanganate ($KMnO_4$) procedure as described by Subbiah and Asija (1956). Available soil P was determined by using Olsen's method (Olsen *et al*., 1954), whereas the soil available K was determined by extracting soil sample with 1 *M* ammonium acetate (CH_3COONH_4) method (Hanway and Heidel, 1952) and taking reading on flame photometer. The organic carbon content in soil samples was determined by Walkley and Black (1934) method. The pH of the soil was determined by Schofield and Taylor (1955) method.

2.14. Plant analysis

Plant samples collected at harvest were dried in hot air oven at 60±2°C for 6 hours. The oven-dried samples of plants and air-dried sample of grains were ground to pass through 40 mesh sieve in a Macro-Wiley Mill. From each replication 0.5 g grain and straw samples were taken for chemical analyses to determine the N, P, and K concentrations.

2.14.1.Determination of total N concentration in plant

The N concentration in wheat grain and straw samples was determined by the modified Kjeldahl method. The method has two main steps:

(i) Digestion of the sample to convert the N compound in sample to NH_4^+ form and

(ii) Determination of NH_4^+ in digested samples by titration with an acid.

(i) Digestion

The digestion of sample was done according to modified version of Kjeldahl procedure (Prasad *et al*., 2006). Accurately weighed 0.5 g of finely ground grain or straw sample was placed in a digestion flask of 100 ml capacity. In each flask, 3-4 g of catalyst mixture (anhydrous sodium sulphate and copper sulphate pentahydrate in 10:1 ratio) and 10 ml concentrated H_2SO_4 were added. Samples were digested till digest got clean aliquot on a digestion unit.

(ii) Distillation and titration

The digested material was transferred into vacuum jacket of Macro-Kjeldahl distillation apparatus. 20 ml of 4% boric acid solution was taken in a conical flask

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containing bromocresol green and methyl red mixed indicator to which the condenser outlet of the flask was dipped. In the Kjeldahl flask, 100 ml of 40% NaOH solution was added. The released ammonia trapped in the boric acid was titrated against 0.02 *N* H_2SO_4. A blank was also carried out simultaneously and titrated against the same acid. The nitrogen concentration of the sample was estimated as:

Amount of N in samples (S) =

$$\frac{(ml\ of\ acid\ used\ for\ sample - ml\ of\ acid\ used\ for\ blank)}{1000}$$ x 14 x Normality of acid

N (%) in samples = S x $$\frac{100}{Weight\ of\ sample\ (g)}$$

2.14.1.1. Crud protein

Crud protein content in wheat grain was computed by multiplying N concentration with a factor of 5.85 (Hussain, 2013)

2.14.1.2. N uptake

N uptake in grain and straw was computed by multiplying their yields with corresponding values of N concentration in them and expressed in kg ha^{-1}. The total N uptake was determined by adding the N uptake by grain and straw for each treatment.

2.14.1.3. Nitrogen use efficiency indices

2.14.1.3.1. Agronomic efficiency (AE)

Agronomic efficiency (AE) denotes the units of additional crop produced per unit of input (nutrient) added externally. It is expressed as kg yield increase/kg of a nutrient added. It has a direct bearing on better utilization of applied nutrients, the profitability of their usage and minimization of nutrient losses. It is computed by using the following formula-

$$AE = \frac{\text{Grain yield in treated plot (kg/ha)} - \text{Grain yield in control plot (kg/ha)}}{\text{Amount of nutrient applied (kg/ha)}}$$

2.14.1.3.2. Recovery efficiency (RE)

Recovery efficiency (RE) is the proportion of applied nutrient taken up by the crop usually expressed as a percentage. Recovery efficiency is calculated with the help of the formula-

$$RE = \frac{\text{nutrient uptake in treated plot (kg/ha)} - \text{nutrient uptake in control plot (kg/ha)}}{\text{Amount of nutrient applied (kg/ha)}} \text{ x } 100$$

2.14.1.3.3. Physiological efficiency (PE)

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Physiological efficiency (PE) refers to the units of crop produced or growth in biomass registered per unit of nutrient absorbed by the crop. It represents the ability of a plant to transform nutrient acquired from fertilizer into economic yield. It is expressed as kg yield increase per kg increase in nutrient uptake from fertilizer. It is calculated by the formula

$$\text{PE} = \frac{\text{Grain yield in treated plot (kg/ha)} - \text{Grain yield in control plot (kg/ha)}}{\text{nutrient uptake in treated plot (kg/ha)} - \text{nutrient uptake in control plot (kg/ha)}}$$

3.14.2 Phosphorus and potassium analysis in plant

Total phosphorus (P) and potassium (K) in wheat samples (grain and straw) were estimated at harvest by Vanadomolybdo phosphoric acid yellow colour method and flame photometry method, respectively on a sulphuric-nitric perchloric tri-acid digest of plant material (Prasad *et al.*, 2006). The P and K concentrations in wheat straw and grain were expressed in percentages.

3.14.2.1 Phosphorus and potassium uptake

Phosphorus (P) and potassium (K) uptake by wheat were computed by multiplying their respective concentrations with dry matter yields (grain and straw) and it was expressed in kg ha^{-1}. The total P or K uptake was determined by adding the P or K uptake by grain and straw for each treatment.

3.15 Statistical analysis

The data relating to each character were analyzed as per the procedure of analysis of variance and significance of a randomized block design and significance tested by "*F*" test (Gomez and Gomez, 1984). Standard Error of Means (SEm±) and Least Significance Difference (LSD) at 5% level of significance were worked out for each parameter. The analyses were carried out using Statistical Analysis System SAS 9.3.

3.16 Economics of wheat cultivation

Cost of cultivation of wheat was computed on the basis of prevalent market prices of inputs during crop season. Gross returns were computed on the basis of grain and straw yields and their prevailing market prices during the crop season. The Net returns were obtained by subtracting cost of cultivation from the gross return i.e.

Net returns (₹ ha^{-1}) = Gross returns (₹ ha^{-1}) – cost of cultivation (₹ ha^{-1})

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Benefit: Cost (B: C) ratio was calculated by using following expression:

B: C = Net returns (₹ ha^{-1})/Cost of cultivation (₹ ha^{-1})

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RESULTS

The results of the experiment entitled **"Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)"** are presented in this chapter. Results have been explained by adding suitable tables and graphs, wherever necessary.

3. Plant growth

3.1.1. Plant height

Plant height of wheat was recorded at 30, 60, 90 days after sowing (DAS) and at harvest. Height of the wheat crop increased quadratically with the advancement of growth stages. Plant height of wheat was significantly influenced by application of bacterial and cyanobacterial strains of PGPR. The data pertaining to plant height of wheat are presented in Table 4.1 and depicted in Fig. 4.1. The results showed that the height of wheat was not significantly influenced at 30 DAS across all the treatments. However, at all other growth stages, plant height of wheat was significantly affected by inoculation of bacterial and cyanobacterial strains of PGPR. There was a significant increase in height of wheat in control + Azo + CW1 + PW5 over absolute control across all the stages of wheat growth. Moreover, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments recorded significantly taller plants at all growth stages over 75% N + RPK but it was at par with RNPK treatment. Whereas, there was no significant difference observed in height of wheat in all growth stages amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest plant height of wheat was observed with application of 75% N + RPK + Azo + CW1 + PW5 across all the growth stages.

3.1.2.Total tillers

Number of tillers m^{-2} of wheat were significantly affected by application of bacterial and cyanobacterial strains of PGPR. Data on number of tillers m^{-2} of wheat as influenced by PGPR are presented in Table 4.2. The results showed that number of tillers m^{-2} of wheat was not significantly influenced at 30 days after sowing (DAS) across all the treatments. However, at all other growth stages, number of tillers m^{-2} of wheat was significantly affected by inoculation of PGPR. There was a significant

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increase in number of tillers m^{-2} of wheat in control + Azo + CW1 + PW5 over absolute control. Moreover, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments produced significantly higher number of tillers m^{-2} in wheat at all the growth stages over 75% N + RPK but it was at par with RNPK treatment. Whereas, there was no significant difference observed in number of tillers m^{-2} of wheat at all the growth stages amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest number of tillers m^{-2} of wheat was produced with the use of 75% N + RPK + Azo + CW1 + PW5 across the all growth stages.

Table 4.1 Effect of PGPR on plant height of wheat

	Plant height (cm)				
Treatment	30 DAS	60 DAS	90 DAS	120 DAS	Harvest
Absolute control	19.6	33.8	73.6	79.6	78.4
Control + Azo + CW1 + PW5	19.7	38.3	79.9	83.8	83.8
RNPK	20.3	48.0	91.2	95.2	95.5
75% N + RPK	20.4	42.6	85.5	90.4	90.0
75% N + RPK + Azo	20.8	42.5	85.7	90.4	89.9
75% N + RPK + CW1	20.9	43.0	85.7	90.2	89.4
75% N + RPK + PW5	20.9	42.7	85.7	90.2	89.8
75% N + RPK + Azo + CW1	20.1	48.2	91.2	95.1	95.3
75% N + RPK + Azo + PW5	20.9	47.9	91.3	95.1	95.7
75% N + RPK + CW1 + PW5	20.9	47.8	91.3	95.3	95.4
75% N + RPK + Azo + CW1 + PW5	21.0	48.6	91.4	95.5	95.5
SEm±	0.798	1.3	1.82	1.22	1.72
LSD (P=0.05)	NS	3.83	5.36	3.6	5.07

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K); NS- Non-sig.

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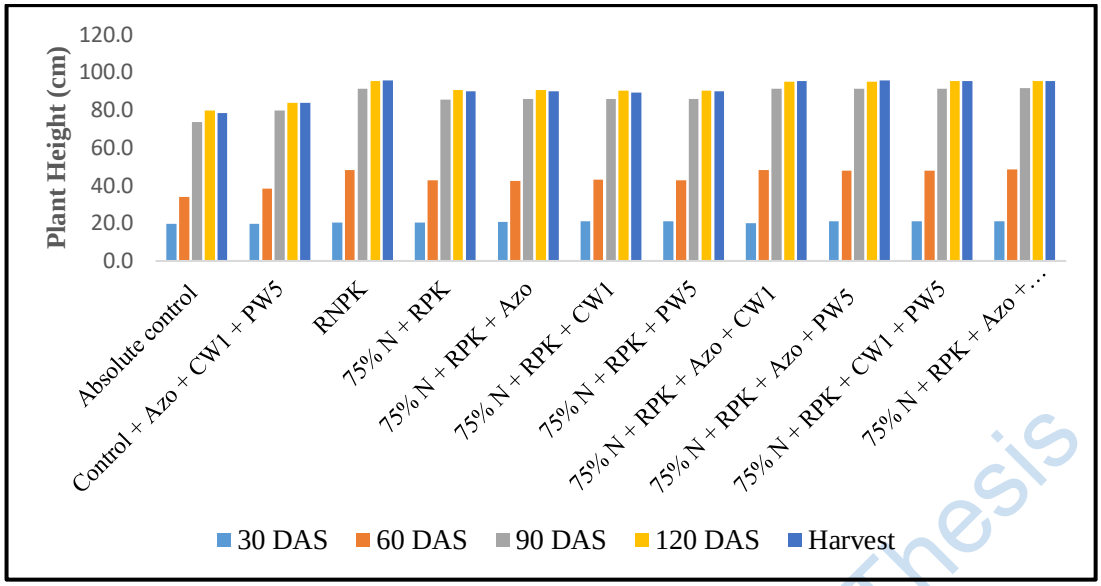

Fig. 4.1 Effect of PGPR on Height of Wheat

Table 4.2 Effect of PGPR on number of tillers of wheat

Treatment	Number of tillers (m^{-2})				
	30 DAS	60 DAS	90 DAS	120 DAS	Harvest
Absolute control	202	378	367	358	349
Control + Azo + CW1 + PW5	205	418	407	398	373
RNPK	211	543	531	523	486
75% N + RPK	206	462	451	442	422
75% N + RPK + Azo	207	470	459	450	422
75% N + RPK + CW1	206	480	469	460	425
75% N + RPK + PW5	215	473	462	453	424
75% N + RPK + Azo + CW1	211	543	532	523	483
75% N + RPK + Azo + PW5	212	536	525	516	479
75% N + RPK + CW1 + PW5	212	541	531	521	481
75% N + RPK + Azo + CW1 + PW5	216	549	538	529	488
SEm±	5.7	13.3	12.9	13.3	8.02
LSD (P=0.05)	NS	39.2	38.2	39.2	23.6

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K); NS- Non-significant

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3.1.3. Dry mater accumulation

Data on dry matter accumulation as influenced by bacterial and cyanobacterial strains of PGPR are presented in Table 4.3. The dry matter accumulation increased progressively and quadratically with the advancement of crop age. It is evident from the Table that dry matter accumulation in shoot of wheat was not significantly influenced at 30 days after sowing (DAS) by different treatments. However, dry matter accumulation in shoot was significantly affected by inoculation of PGPR at all the other growth stages.

There was a significant increase in dry matter production in control + Azo + CW1 + PW5 over absolute control across all the growth stages. Further, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments produced significantly higher dry matter at all the growth stages over 75% N + RPK, but it was at par with RNPK treatment. Whereas, there was no significant difference observed for dry matter production amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest dry matter was produced with application of 75% N + RPK + Azo + CW1 + PW5 across the all-growth stages.

Table 4.3 Effect of PGPR on dry matter accumulation in wheat

	Dry matter accumulation (g m^{-2})				
Treatment	30 DAS	60 DAS	90 DAS	120 DAS	Harvest
Absolute control	18	57	341	669	921
Control + Azo + CW1 + PW5	18	100	407	774	1049
RNPK	19	197	672	1058	1340
75% N + RPK	19	145	495	933	1196
75% N + RPK + Azo	19	151	517	931	1192
75% N + RPK + CW1	19	151	513	929	1197
75% N + RPK + PW5	20	147	510	931	1192
75% N + RPK + Azo + CW1	20	199	679	1070	1355
75% N + RPK + Azo + PW5	20	198	677	1067	1335
75% N + RPK + CW1 + PW5	19	198	673	1061	1321
75% N + RPK + Azo + CW1 + PW5	19	199	678	1069	1358
SEm±	3	14	22	26	37
LSD (P=0.05)	NS	42	64	77	109

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3.1.4.Leaf area index

Data pertaining to leaf area index (LAI) as influenced by bacterial and cyanobacterial strains of PGPR are presented in Table 4.4. Leaf area index (LAI) of wheat measured at 30, 60, and 90 days after sowing (DAS) was significantly influenced due to inoculation of bacterial and cyanobacterial strains of PGPR. At 30 DAS, the treatment 75% N + RPK + Azo + CW1 recorded significantly higher LAI over absolute control and control + Azo + CW1 + PW5, whereas it was at par with all other treatments with respect to the LAI. At 60 and 90 DAS, absolute control produced significantly lower LAI over control + Azo + CW1 + PW5. Moreover, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 treatments recorded significantly higher LAI at 60 and 90 DAS over 75% N + RPK, but it was at par with RNPK treatment. Whereas, there was no significant difference observed for LAI production at 60 and 90 DAS amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest LAI values were recorded with application of 75% N + RPK + Azo + CW1 across all the growth stages.

Table 4.4 Effect of PGPR on leaf area index of wheat

Treatment	LAI		
	30 DAS	60 DAS	90 DAS
Absolute control	0.05	1.82	2.02
Control + Azo + CW1 + PW5	0.07	2.48	2.75
RNPK	0.09	3.91	4.34
75% N + RPK	0.09	3.18	3.54
75% N + RPK + Azo	0.11	3.18	3.54
75% N + RPK + CW1	0.12	3.20	3.56
75% N + RPK + PW5	0.14	3.15	3.50
75% N + RPK + Azo + CW1	0.16	3.93	4.36
75% N + RPK + Azo + PW5	0.14	3.92	4.34
75% N + RPK + CW1 + PW5	0.11	3.92	4.35
75% N + RPK + Azo + CW1 + PW5	0.11	3.92	4.34
SEm±	0.03	0.22	0.24
LSD (P=0.05)	0.09	0.64	0.71

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3.2. Mean crop growth rate

Data on growth rate of wheat as influenced by PGPR are presented in Table 4.5. Growth rate of wheat was significantly affected by application of bacterial and cyanobacterial strains of PGPR. Growth rate of wheat was significantly higher in control + Azo + CW1 + PW5 treatment over absolute control at all the growth stages. Further, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments exhibited significantly higher growth rate in wheat at all the growth stages over 75% N + RPK, but it was at par with RNPK treatment. Whereas, there was no significant difference observed in growth rate of wheat at all the growth stages amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest growth rate of wheat was recorded with application of 75% N + RPK + Azo + CW1 + PW5 across all the growth stages.

Table 4.5 Effect of PGPR on crop growth rate of wheat

Treatment	CGR (g m^{-2} d^{-1})			
	60 DAS	90 DAS	120 DAS	Harvest
Absolute control	2.4	8.7	6.0	6.0
Control + Azo + CW1 + PW5	3.0	10.2	7.5	7.4
RNPK	6.0	16.0	12.2	12.3
75% N + RPK	4.2	11.7	9.5	10.3
75% N + RPK + Azo	4.4	12.2	9.9	10.2
75% N + RPK + CW1	4.4	12.1	10.2	10.1
75% N + RPK + PW5	4.2	12.1	10.1	10.3
75% N + RPK + Azo + CW1	6.0	16.0	12.0	12.0
75% N + RPK + Azo + PW5	5.9	16.0	12.1	12.5
75% N + RPK + CW1 + PW5	6.0	15.8	12.4	12.2
75% N + RPK + Azo + CW1 + PW5	6.2	16.4	13.0	12.9
SEm±	0.49	0.93	0.47	0.52
LSD (P=0.05)	1.45	2.67	1.40	1.54

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

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3.3. Mean relative growth rate

Relative growth rate of wheat was significantly affected by application of bacterial and cyanobacterial strains of PGPR (Table 4.6). The results suggested that relative growth rate of wheat was not significantly influenced during 60 to 90 days after sowing (DAS) between absolute controls and control + Azo + CW1 + PW treatments, but it was significantly higher during 90 DAS to harvest in control + Azo + CW1 + PW5 over absolute control. Additionally, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments showed significantly higher relative growth rate in wheat during all the growth stages over 75% N + RPK, but it was at par with RNPK treatment. Whereas, there was no significant difference observed in relative growth rate of wheat in all growth stages amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest growth relative rate of wheat was recorded with application of 75% N + RPK + Azo + CW1 + PW5 across the all growth stages.

Table 4.6 Effect of PGPR on relative growth rate of wheat

Treatment	RGR (mg g^{-1} dry matter d^{-1})			
	60 DAS	90 DAS	120 DAS	Harvest
Absolute control	53.1	55.0	25.0	19.2
Control + Azo + CW1 + PW5	54.0	55.7	30.9	23.8
RNPK	64.2	68.7	38.2	30.0
75% N + RPK	59.6	63.8	35.5	27.3
75% N + RPK + Azo	61.2	65.3	36.3	27.9
75% N + RPK + CW1	60.4	64.8	36.0	27.7
75% N + RPK + PW5	60.2	64.5	35.8	27.6
75% N + RPK + Azo + CW1	65.6	70.2	39.0	30.0
75% N + RPK + Azo + PW5	64.7	69.3	38.5	29.6
75% N + RPK + CW1 + PW5	64.3	68.9	38.3	29.5
75% N + RPK + Azo + CW1 + PW5	66.5	71.2	39.6	30.4
SEm±	1.72	0.98	0.62	0.47
LSD (P=0.05)	5.12	2.89	1.83	1.39

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Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

3.4. Mean net assimilation rate

Data on net assimilation rate of wheat as influenced by bacterial and cyanobacterial strains of PGPR are given in Table 4.7. The results showed that net assimilation rate was significantly affected by application of PGPR. Net assimilation rate of wheat was significantly higher in control + Azo + CW1 + PW5 treatment over absolute control at all the growth stages. Furthermore, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments recorded significantly higher net assimilation rate in wheat at all the growth stages over 75% N + RPK, but it was at par with RNPK treatment. Whereas, there was no significant difference observed in net assimilation rate of wheat at all the growth stages amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK treatments. The highest net assimilation rate of wheat was recorded with application of 75% N + RPK + CW1 + PW5 across all the growth stages.

Table 4.7 Effect of PGPR on net assimilation rate of wheat

Treatment	NAR (g m^{-2} leaf area d^{-2})	
	60 DAS	90 DAS
Absolute control	1.02	1.02
Control + Azo + CW1 + PW5	2.75	2.75
RNPK	6.84	6.84
75% N + RPK	4.82	4.82
75% N + RPK + Azo	4.76	4.76
75% N + RPK + CW1	4.73	4.73
75% N + RPK + PW5	4.66	4.66
75% N + RPK + Azo + CW1	6.72	6.72
75% N + RPK + Azo + PW5	6.77	6.77
75% N + RPK + CW1 + PW5	6.90	6.90
75% N + RPK + Azo + CW1 + PW5	6.76	6.76
SEm±	0.59	0.13
LSD (P=0.05)	1.73	0.39

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Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

3.5. Yield attributes

Data pertaining to yield attributes as influenced by PGPR are presented in Table 4.8. Yield attributes of wheat were significantly affected by application different combination of bacterial and cyanobacterial strains of PGPR.

The results indicated that effective tillers were significantly influenced by application of PGPR. Absolute control recorded significantly lower effective tillers than control + Azo + CW1 + PW5. There was a significant increase with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK with respect to the effective tillers, whereas they were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, and 75% N + RPK+ PW5 recorded significantly lower values for effective tillers over RNPK, but they were at par with 75% N + RPK. The highest effective tillers were recorded for application of 75% N + RPK + Azo + CW1 + PW5 treatment.

The results showed that spike length was significantly influenced by application of PGPR. Spikes were significantly shorter in absolute control as compared to control + Azo + CW1 + PW5. There was a significant increase in spike length with the application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK, whereas all the former treatments were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 recorded significantly lower spike length over RNPK, but they were at par with 75% N + RPK. The highest spike length was recorded with the application of 75% N + RPK + Azo + PW5.

The results showed that spike weight was significantly influenced by application of PGPR. Absolute control recorded significantly lower spike weight than control + Azo + CW1 + PW5. There was a significant increase with the application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK with respect to the spike weight, whereas it was at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 recorded

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significantly lower spike weight over RNPK, but all the former treatments were at par with 75% N + RPK. The highest spike weight was recorded with application of 75% N + RPK + Azo + CW1 + PW5.

Grain weight per spike was significantly influenced by application of PGPR. Absolute control recorded significantly lower grain weight per spike than control + Azo + CW1 + PW5. There was a significant increase in grain weight per spike with the application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK, whereas all the former treatments were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 recorded significantly lower grain weight per spike over RNPK, but they were at par with 75% N + RPK. The highest values for grain weight per spike were recorded for application of 75% N + RPK + Azo + CW1 + PW5.

The results showed that number of grains per spike was significantly influenced by application of PGPR. Absolute control recorded significantly lower number of grains per spike than control + Azo + CW1 + PW5. There was significant increase with the application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK with respect to the number of grains per spike, whereas they were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 recorded significantly lower values for number of grains per spike over RNPK, but they were at par with 75% N + RPK. The highest number of grains per spike was recorded with application of 75% N + RPK + CW1 + PW5.

Test weight was significantly influenced by application of PGPR. Absolute control recorded significantly lower test weight than control + Azo + CW1 + PW5. Significant increase was observed with the application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK with respect to the test weight, whereas they were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 recorded significantly lower test weight over RNPK, but they were at par with 75% N + RPK. The highest values for test weight was recorded with application of 75% N + RPK + Azo + CW1 and 75% N + RPK + Azo + PW5 treatments.

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Table 4.8 Effect of PGPR on yield attributes of wheat

Treatment	Effective tillers m^{-2}	Spike length (cm)	Spike weight (g)	Grain weight (g) $spike^{-1}$	No. of grains $spike^{-1}$	Test weight (g)
Absolute control	343	10.0	2.0	1.39	30	30.3
Control + Azo + CW1 + PW5	368	10.5	2.2	1.68	36	34.0
RNPK	481	12.0	3.0	2.47	50	42.0
75% N + RPK	417	11.2	2.5	2.00	44	37.2
75% N + RPK + Azo	417	11.1	2.6	2.06	44	37.0
75% N + RPK + CW1	420	11.1	2.5	2.09	44	37.7
75% N + RPK + PW5	419	11.1	2.5	2.04	44	37.7
75% N + RPK + Azo + CW1	478	12.0	3.0	2.40	50	42.3
75% N + RPK + Azo + PW5	474	12.1	3.0	2.45	50	42.3
75% N + RPK + CW1 + PW5	476	12.0	3.0	2.45	52	42.0
75% N + RPK + Azo + CW1 + PW5	483	12.0	3.2	2.46	50	41.0
SEm±	8.02	0.093	0.08	0.08	1.95	0.96
LSD (P=0.05)	23.7	0.27	0.24	0.24	5.76	2.84

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

3.5.1. **Root Growth**

Application of bacterial and cyanobacterial strains of PGPR on wheat has significantly influenced the root growth. Data on the root growth as influenced by PGPR are presented in Table 4.9. The results showed that 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 have recorded significantly higher root length, volume and dry weight over the application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK, 75% N + RPK. Further, control + Azo + CW1 + PW5 and absolute control produced significantly lower values over all other treatment with respect to root length, volume and dry weight. The highest root length of 4.08 cm cm-

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^{3} of soil, root volume of 6.78 mm^3 cm^{-3} of soil and dry weight 0.93 mg cm^{-3} of soil were obtained from application of 75% N + RPK + Azo + CW1 + PW5.

Table 4.9 Effect of PGPR on root growth of wheat

Treatment	Root length (cm) cm^{-3} of soil	Root volume (mm^3) cm^{-3} of soil	Root dry weigh (mg) cm^{-3} of soil
Absolute control	3.02	3.93	0.36
Control + Azo + CW1 + PW5	3.09	4.10	0.39
RNPK	3.57	5.20	0.61
75% N + RPK	3.48	5.43	0.60
75% N + RPK + Azo	3.60	5.57	0.62
75% N + RPK + CW1	3.59	5.63	0.63
75% N + RPK + PW5	3.62	5.10	0.62
75% N + RPK + Azo + CW1	4.01	6.54	0.88
75% N + RPK + Azo + PW5	4.03	6.61	0.87
75% N + RPK + CW1 + PW5	4.05	6.51	0.89
75% N + RPK + Azo + CW1 + PW5	4.08	6.78	0.93
SEm±	0.12	0.29	0.06
LSD (P=0.05)	0.36	0.85	0.17

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

3.6. Biological, grain and straw yields, and harvest index

Data on biological, grain and straw yields, and harvest index are given in Table 4.10. and depicted in Fig. 4.2.

The data from analysis of variance indicated that both biological and straw yields were significantly influenced with the application of PGPR. Data showed that combined application of PGPR species (Azo + CW1 + PW5) alone significantly enhanced the biological and straw yields of wheat as compared to the absolute control. The inoculation of PGPR increased biological and straw yields by 10% over absolute control.

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Further, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 treatments produced significantly higher biological and straw yields over 75% N + RPK treatments, but they were at par with RNPK. In addition, application of 75% N + RPK produced significantly lower biological and straw yields over application of recommended dose of NPK fertilizes, however the former was at par with application of 75% N and inoculation of any one of the three PGPR species (75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5).The data suggested that the highest biological yields were recorded with combined inoculation of all the three PGPR species (75% N + RPK + Azo + CW1 + PW5). It was followed by 75% N + RPK + Azo + PW5 treatment. However the highest straw yield was obtained with application of 75% N + RPK + Azo + PW5. Treatments 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + PW5, 75% N + RPK + Azo + CW1 and RNPK gave 16, 15, 15,15 and 15% higher biological yield, respectively, over 75% N + RPK. Similarly, treatments 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + PW5, 75% N + RPK + Azo + CW1 and RNPK gave 12.7, 12.7, 13.2, 12.3 and 12.% higher straw yields, respectively, over 75% N + RPK. The grain yield of wheat was influenced significantly by application of PGPR. Data indicated that combined application of three PGPR (Azo + CW1 + PW5) alone increased the grain yield of wheat significantly over the absolute control (no PGPR). The increase in grain yield by the former treatment was 10.3% over the later. Hence, the response of PGPR was significant when three species of PGPR were inoculated together even without NPK fertilizers application.

It is evident from the data that the highest grain yield was recorded when 75% N was combined with RPK and all the three species of PGPR tested (75% N + RPK + Azo + CW1 + PW5). The next best treatments, with respect to grain yield, were combinations of 75% N + RPK either with any two species of PGPR, i.e. 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5. Combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar grain yields, all being at par with recommended dose of NPK (RNPK). There was a significant reduction in grain yield if only one species (Azo, CW1 or PW5) or no species (75% N + RPK) of PGPR was combined with 75% N + RPK as compared to all the former treatments. Treatments 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and

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RNPK gave 22.7, 19.3, 17.9, 17.9 and 18.4% higher grain yield, respectively, over 75% N + RPK.

It is thus clear that a significant grain yield response to PGPR inoculation was achieved in the present study. This response was achieved in both situations of no NPK or NPK application. The grain yield increased more profusely if all the three species of PGPR were combined with 75% N + RPK. Interestingly, the grain yield increase was similar when either two or three species of PGPR were combined with 75% N + RPK. The combined use of either two or three species of PGPR with 75% N + RPK was equally effective in increasing the wheat grain yield as application of recommended dose of fertilizers (RNPK). Thus these biofertilizers could help in saving the nitrogenous fertilizers in wheat production. In addition, application of 75% N + RPK produced significantly lower biological and grain yields over RNPK, however the former was at par with application 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5.

The data indicated that harvest index of what was significantly influenced with application of PGPR. Data showed that combined application of PGPR species (Azo + CW1 + PW5) alone did not significantly influence the harvest index as compared to absolute control. There was no significant difference observed with application of 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, RNPK 75% N + RNPK, but the former treatments gave significantly higher harvest index over all the other treatments. In addition the 75% N + RPK, 75% N + RPK + Azo, 75% N + RPK + CW1 and 75% N + RPK+ PW5 did not differ significantly among themselves with respect to harvest index. The highest harvest index was obtained with the application of 75% N + RPK + Azo + CW1 + PW5 and it was followed by 75% N + RPK + Azo + CW1.

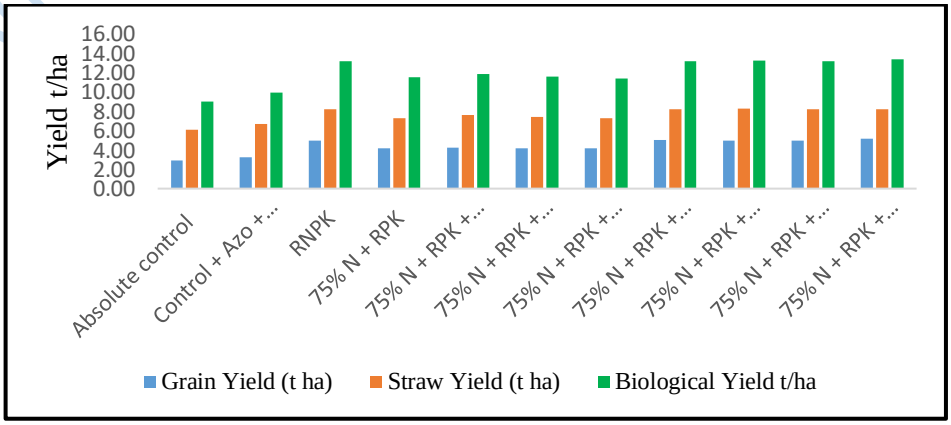

Fig 4.2 Effect of PGPR on grain yield, straw yield, and biological yield of wheat

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Table 4.10 Effect of PGPR on grain yield, straw yield, biological yield and harvest index of wheat

Treatment	Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	Biological yield (t ha^{-1})	Harvest index (%)
Absolute control	2.91	6.10	9.01	32.3
Control + Azo + CW1 + PW5	3.21	6.69	9.90	32.5
RNPK	4.96	8.23	13.19	37.6
75% N + RPK	4.19	7.30	11.49	36.5
75% N + RPK + Azo	4.21	7.63	11.84	35.5
75% N + RPK + CW1	4.15	7.43	11.59	35.9
75% N + RPK + PW5	4.14	7.27	11.40	36.3
75% N + RPK + Azo + CW1	5.00	8.20	13.20	37.9
75% N + RPK + Azo + PW5	4.94	8.27	13.21	37.4
75% N + RPK + CW1 + PW5	4.94	8.23	13.17	37.5
75% N + RPK + Azo + CW1 + PW5	5.14	8.23	13.38	38.5
SEm±	0.07	0.18	0.18	0.72
LSD (P=0.05)	0.21	0.54	0.53	2.13

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

3.9. Correlation studies between yield and yield attributes

Yield production in cereals is a complex coordinated process that include formation and subsequent re-assimilation of yield components. These processes are governed by the genetic characteristics and strongly affected by environmental conditions. This is shown by positive correlation between different yield attributes and grain yield (Fig.4.3). The R^2 values between grain yield and different yield attributes such as effective tillers m^{-2}, spike length, spike weight, number of grains $spike^{-1}$, grain weight $spike^{-1}$ and test weight were 0.74, 0.90, 0.88, 0.88, 0.89 and 0.84 respectively. It suggests that, for example, 74% of the variation in mean grain yield of wheat could be adequately explained by the regression equation computed ($y = 0.0089x + 0.6169$) between grain yield and effective tillers. Similarly, 89% of the variation in mean grain

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yield of wheat could be adequately explained by the regression equation computed (y = 1.8933x + 0.3019) between grain yield and grain weight /spike.

4. Microbial biomass carbon

5. The data related to soil microbial biomass carbon measured at flowering stage of wheat are presented in Table 4.11. Data indicated that soil microbial biomass carbon was significantly influenced with application of bacterial and cyanobacterial stains of PGPR. Combined application of three PGPR species (Azo + CW1 + PW5) alone has significantly increased soil microbial biomass carbon over absolute control. Further, it was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 showed significantly higher microbial biomass carbon over both RNPK and 75% N + RPK treatments. Whereas, there was no significant difference observed for the soil microbial biomass carbon among 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK and 75% N + RPK treatment. The highest microbial biomass carbon was obtained with application of 75% N + RPK + Azo + CW1 + PW5.

Data pertaining to dehydrogenase enzyme activity measured at flowering stage of wheat are presented in Table 4.11. Data showed that dehydrogenase activity was significantly influenced with application of bacterial and cyanobacterial stains of PGPR. Combined application of three PGPR species (Azo + CW1 + PW5) alone has significantly increased the dehydrogenase enzyme activity over absolute control. Moreover, it was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 showed significantly higher dehydrogenase enzyme activity over both RNPK and 75% N + RPK treatments. Whereas, there was no significant difference observed for dehydrogenase activity amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK and 75% N + RPK treatment. The highest dehydrogenase activity was obtained with application of 75% N + RPK + Azo + CW1 + PW5.

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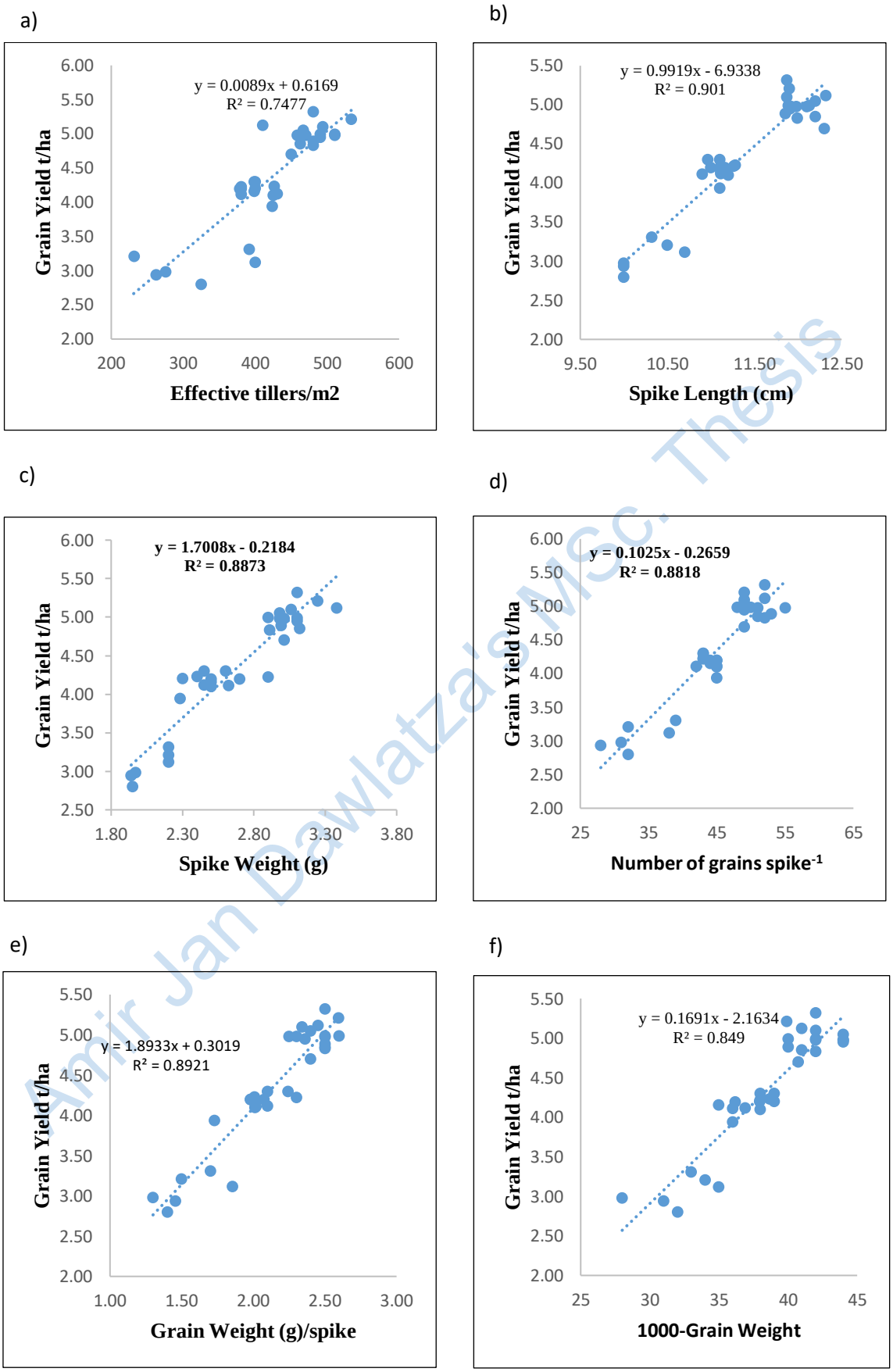

Fig. 4.3 Correlation between yield attributes and gain yield

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5.2. Soil chlorophyll

The data pertaining to soil chlorophyll measured at flowering stage of wheat are presented in Table 4.11. Data showed that soil chlorophyll content was significantly influenced with application of bacterial and cyanobacterial stains of PGPR. Combined application of three PGPR species (Azo + CW1 + PW5) alone has PGPR. Application of PGPR significantly increased soil chlorophyll content over absolute control. It was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 had significantly higher soil chlorophyll content over both RNPK and 75% N + RPK treatments. Whereas, there was no significant difference observed for soil chlorophyll among 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK and 75% N + RPK treatment. The highest soil chlorophyll content was obtained from application of 75% N + RPK + Azo + CW1 + PW5.

5.3. Fluorescein diacetate hydrolysis

Data pertaining to fluorescein diacetate (FDA) hydrolysis analyzed at flowering stage of wheat are presented in Table 4.11. The data indicated significant difference for application of different bacterial and cyanobacterial strains of PGPR. Application of 75% N + RPK + Azo + CW1 and 75% N + RPK + Azo + PW5 showed significantly highest values for FDA hydrolysis over all the other treatments. Whereas, RNPK treatment showed significantly lower values for FDA hydrolysis than 75% N + RPK + Azo, 75% N + RPK + Azo + CW1 and 75% N + RPK + Azo + PW5, and on the other hand it was at par with 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + CW1 and 75% N + RPK+ PW5. The highest values for FDA hydrolysis were recorded with application of 75% N + RPK + Azo + PW5.

5.4. Concentration and uptake of nitrogen (N) in grain and straw and protein content in grain

Data pertaining to N concentration and uptake in grain and straw and protein content in grain are presented in Table 4.12. Concentration and uptake of N in grain and straw, and protein content in grain were significantly influenced with the application of bacterial and cyanobacterial strains of PGPR. The results showed that N concentration and uptake in grain and straw, and protein content in grain were significantly lower in absolute than inoculation of three species of PGPR (Azo + CW1 + PW5) alone.

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Table 4.11 Effect of PGPR on soil microbial parameters at flowering stage of wheat

Treatments	MBC (µg g^{-1} of soil)	Soil chlorophyll (µg g^{-1})	Dehydrogenase activity (µg g^{-1} soil day^{-1})	FDA hydrolysis (µg F. g^{-1} soil h^{-1})
Absolute control	654	0.65	21.7	0.012
Control + Azo + CW1 + PW5	700	0.70	30.7	0.015
RNPK	747	0.75	38.3	0.012
75% N + RPK	767	0.77	40.2	0.018
75% N + RPK + Azo	757	0.76	43.0	0.018
75% N + RPK + CW1	752	0.75	41.0	0.015
75% N + RPK + PW5	750	0.75	40.3	0.015
75% N + RPK + Azo + CW1	820	0.82	52.4	0.019
75% N + RPK + Azo + PW5	826	0.83	51.7	0.020
75% N + RPK + CW1 + PW5	823	0.82	50.8	0.015
75% N + RPK + Azo + CW1 + PW5	837	0.84	52.4	0.015
SEm±	15	0.01	2.291	0.001
LSD (P=0.05)	45	0.04	6.8	0.003

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

Further, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 treatments significantly increased N concentration and uptake in grain and straw, and protein content in grain over 75% N + RPK, whereas the former treatments were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 did not significantly increase N concentration and uptake in grain and straw, and protein content in grain over RNPK, however, they were at par with application of 75% N + RPK. The highest values for N concentration and uptake in grain and straw, and protein content in grain were obtained with application of 75% N + RPK + Azo + CW1 + PW5.

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Table 4.12 Effect of PGPR on nitrogen (N) concentration and uptake in wheat

Treatment	N conc. in grain (%)	Protein content (%)	N conc. in straw (%)	N uptake in grain kg ha^{-1}	N uptake in straw kg ha^{-1}	Total N uptake kg ha^{-1}
Absolute control	1.17	9.95	0.247	49.5	15.0	64.5
Control + Azo + CW1 + PW5	1.50	10.53	0.350	57.8	23.4	81.2
RNPK	2.22	12.97	0.517	109.8	42.5	152.3
75% N + RPK	1.83	10.73	0.460	77.0	33.7	110.7
75% N + RPK + Azo	2.07	12.13	0.517	87.2	39.5	126.7
75% N + RPK + CW1	2.19	12.79	0.547	90.9	40.5	131.5
75% N + RPK + PW5	2.06	12.03	0.517	85.1	37.5	122.6
75% N + RPK + Azo + CW1	2.45	14.33	0.613	122.5	50.3	172.8
75% N + RPK + Azo + PW5	2.46	14.37	0.613	121.4	50.7	172.1
75% N + RPK + CW1 + PW5	2.46	14.41	0.616	121.7	50.7	172.4
75% N + RPK + Azo + CW1 + PW5	2.47	14.39	0.617	126.5	50.8	177.4
SEm±	0.10	0.636	0.033	4.747	2.467	6.676
LSD (P=0.05)	0.3	1.9	0.1	14.0	7.3	19.6

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

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5.5. Phosphorus (P) concentration and uptake in grain and straw

Data pertaining to P concentration in grain and straw and its uptake as influenced by inoculation of bacterial and cyanobacterial strains of PGPR are presented in Table 4.13. Concentration and uptake of P both in grain and straw were significantly influenced by inoculation of PGPR. The data showed that P concentration in grain and straw and its respective uptake was significantly higher in 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 and RNPK treatments over 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, absolute control and control + Azo + CW1 +

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PW5 treatments. The highest P concentration in grain and straw, and its respective uptake were recorded with application of 75% N + RPK + Azo + CW1 + PW5

Table 4.13 Effect of PGPR on phosphorus (P) concentration and uptake in wheat

Treatment	P conc. in grain (%)	P conc. in straw (%)	P uptake in grain kg ha^{-1}	P uptake in straw kg ha^{-1}	Total P uptake kg ha^{-1}
Absolute control	0.51	0.021	14.9	1.3	16
Control + Azo + CW1 + PW5	0.59	0.024	19.0	1.6	21
RNPK	0.76	0.031	37.9	2.6	40
75% N + RPK	0.60	0.024	25.1	1.8	27
75% N + RPK + Azo	0.61	0.025	26.0	1.9	28
75% N + RPK + CW1	0.60	0.024	25.0	1.8	27
75% N + RPK + PW5	0.61	0.025	25.2	1.8	27
75% N + RPK + Azo + CW1	0.79	0.032	39.1	2.6	42
75% N + RPK + Azo + PW5	0.77	0.031	37.9	2.6	41
75% N + RPK + CW1 + PW5	0.77	0.031	38.3	2.6	41
75% N + RPK + Azo + CW1 + PW5	0.85	0.034	43.7	2.8	47
SEm±	0.05	0.002	2.48	0.182	2.63
LSD (P=0.05)	0.15	0.006	7.32	0.530	7.78

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

5.6. Potassium (K) concentration and uptake in grain and straw

The data pertaining to K concentration and uptake in grain and straw are presented in Table 4.14. Concentration of K in grain and straw were not significantly influenced with application of bacterial and cyanobacterial strains of PGPR, however, the uptake of K in grain and straw was significantly influenced. There was no significant difference between absolute control and application of only Azo + CW1 + PW5 with respect K uptake in grain and straw. The uptake of K in grain was significantly higher with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5

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and RNPK over application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 and 75% N + RPK. In addition, the uptake of K in straw was significantly higher with application of 75% N + RPK + Azo, 75% N + RPK + CW1, RNPK, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments over application of 75% N + RPK and 75% N + RPK+ PW5. Application of 75% N + RPK + Azo + CW1 + PW5 showed highest values for uptake of K both in grain and straw.

Table 4.14 Effect of PGPR on potassium (K) concentration and uptake in wheat

Treatment	K conc. in grain (%)	K conc. in straw (%)	K uptake in grain kg ha^{-1}	K uptake in straw kg ha^{-1}	Total K uptake kg ha^{-1}
Absolute control	0.317	1.62	9.21	98.6	107.8
Control + Azo + CW1 + PW5	0.317	1.62	10.16	107.9	118.1
RNPK	0.327	1.67	16.20	137.1	153.3
75% N + RPK	0.320	1.63	13.42	119.3	132.7
75% N + RPK + Azo	0.327	1.67	13.75	127.3	141.1
75% N + RPK + CW1	0.330	1.68	13.71	125.0	138.7
75% N + RPK + PW5	0.320	1.63	13.24	118.6	131.8
75% N + RPK + Azo + CW1	0.320	1.63	15.99	133.9	149.9
75% N + RPK + Azo + PW5	0.330	1.68	16.28	139.3	155.5
75% N + RPK + CW1 + PW5	0.330	1.68	16.29	138.9	155.2
75% N + RPK + Azo + CW1 + PW5	0.340	1.73	17.48	142.8	160.3
SEm±	0.011	0.058	0.554	6.223	6.644
LSD (P=0.05)	NS	NS	1.63	18.4	19.6

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

5.7. Available nitrogen (N), phosphorus (P), potassium (K) and organic Carbon in soil

The data pertaining to available N, P, K and organic carbon analyzed at the harvest stage of wheat are presented in Table 4.15. The data showed that the

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application of bacterial and cyanobacterial stains of PGPR did not show a significant difference with respect to soil organic carbon, however, available N, P and K in soil were influenced significantly. Absolute control and application of only Azo + CW1 + PW5 recorded significantly lower values for available N, P, and K in soil over all the other treatments.

5.8. **Nitrogen use efficiency of wheat**

The data pertaining to agronomic efficiency of wheat are presented in Table 4.16. The results from analysis of variance showed that agronomic efficiency of N was significantly influenced by application of bacterial and cyanobacterial strains of PGPR. Agronomic efficiency was significantly higher with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 over RNPK, 75% N + RPK, 75% N + RPK + Azo, 75% N + RPK + CW1, and 75% N + RPK+ PW5 treatments. Further RNPK treatment showed significantly higher agronomic efficiency over 75% N + RPK, 75% N + RPK + Azo, 75% N + RPK + CW1, and 75% N + RPK+ PW5 treatments. The highest agronomic efficiency was obtained with the application of 75% N + RPK + Azo + CW1 + PW5 treatment.

The data pertaining to recovery efficiency of wheat are presented in Table 4.16. The results from analysis of variance indicated that recovery efficiency of nitrogen was significantly influenced by application of bacterial and cyanobacterial strains of PGPR. Recovery efficiency was significantly higher in combined application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 over RNPK, 75% N + RPK, 75% N + RPK + Azo, 75% N + RPK + CW1, and 75% N + RPK+ PW5 treatments. Further, RNPK treatment showed significantly higher recovery efficiency over 75% N + RPK, 75% N + RPK + Azo, 75% N + RPK + CW1, and 75% N + RPK+ PW5 treatments. The highest recovery efficiency was obtained with application of 75% N + RPK + Azo + CW1 + PW5 treatment.

The data pertaining to physiological efficiency of wheat are presented in Table 4.16. The results from analysis of variance suggested that physiological efficiency of nitrogen was significantly influenced by application of bacterial and cyanobacterial strains of PGPR. Application of 75% N + RPK showed the highest physiological efficiency over all other treatment, except it was at par with application of RNPK treatment. Further there was no significant difference observed between application of

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RNPK treatment and 75% N + RPK+ PW5 treatment. The highest physiological efficiency was obtained with application of 75% N + RPK treatment.

Table 4.15 Effect of PGPR on soil organic carbon (OC) , available nitrogen (N), phosphorus (P) and potassium (K) of wheat soil at harvest

Treatment	OC (%)	N kg ha^{-1}	P ha^{-1}	K ha^{-1}
Absolute control	0.50	103.3	7.0	244
Control + Azo + CW1 + PW5	0.52	107.7	7.6	246
RNPK	0.51	170.3	11.6	268
75% N + RPK	0.51	164.3	11.2	266
75% N + RPK + Azo	0.54	168.0	11.9	265
75% N + RPK + CW1	0.49	167.3	11.2	264
75% N + RPK + PW5	0.51	169.0	11.3	265
75% N + RPK + Azo + CW1	0.49	171.7	12.5	264
75% N + RPK + Azo + PW5	0.51	170.0	11.8	266
75% N + RPK + CW1 + PW5	0.51	170.3	12.7	264
75% N + RPK + Azo + CW1 + PW5	0.53	170.3	12.3	271
SEm±	0.023	1.636	0.928	3.022
LSD (P=0.05)	0.07	4.82	2.74	8.91

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

Table 4.16 Effect of PGPR on nitrogen use efficiency of wheat

Treatment	Agronomic efficiency (kg grain increase kg N applied	Recovery efficiency (N kg increase in N uptake per kg N applied	Physiological efficiency (kg grain/ N absorbed)
Control + Azo + CW1 + PW5	-		17.5
RNPK	13.7	58.6	23.3
75% N + RPK	11.4	41.1	30.0
75% N + RPK + Azo	11.5	55.3	20.9

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75% N + RPK + CW1	11.1	59.5	18.8
75% N + RPK + PW5	10.9	51.7	21.2
75% N + RPK + Azo + CW1	18.6	96.3	19.4
75% N + RPK + Azo + PW5	18.1	95.7	19.0
75% N + RPK + CW1 + PW5	18.1	95.9	18.9
75% N + RPK + Azo + CW1 + PW5	19.9	100.3	19.9
SEm±	0.64	4.80	2.5
LSD (P=0.05)	1.93	14.39	7.5

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp*.; PW5- *Providencia sp*.; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

5.9. Economics of wheat cultivation

Data pertaining to gross return, net return, and B: C ratio of wheat as influenced by application of bacterial and cyanobacterial stains of PGPR are presented in Table 4.17. The application of bacterial and cyanobacterial strains significantly influenced gross return, net return, and benefit cost ratio. The results indicated that there was no significant difference with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 and RNPK in gross return, net return and benefit cost ratio, but they were significantly higher from 75% N + RPK. However, highest gross return (₹ 90,500 ha^{-1}), net return (₹ 62,500 ha^{-1}) and benefit cost ratio (2.17) were obtained by application of 75% N + RPK + Azo + CW1 + PW5. The lowest gross return (₹ 56,200 ha^{-1}) and net return (₹ 32,000 ha^{-1}) and benefit cost ratio (1.32) were achieved with absolute control.

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Table 4.17 Effect of different treatments on economics of PGPR inoculation in wheat

Treatment	Gross return ($x10^3$ ₹ ha-1)	Cost of cultivation ($x10^3$ ₹ ha-1)	Net return ($x10^3$ ₹ ha-1)	B : C
Absolute control	56.2	24.20	32.0	1.32
Control + Azo + CW1 + PW5	62.0	24.67	37.3	1.51
RNPK	88.3	28.25	60.1	2.13
75% N + RPK	75.9	27.57	48.3	1.75
75% N + RPK + Azo	77.2	27.89	49.3	1.77
75% N + RPK + CW1	75.9	27.89	48.0	1.72
75% N + RPK + PW5	75.1	27.89	47.2	1.69
75% N + RPK + Azo + CW1	88.7	27.97	60.7	2.17
75% N + RPK + Azo + PW5	88.2	27.97	60.2	2.15
75% N + RPK + CW1 + PW5	88.1	27.97	60.1	2.15
75% N + RPK + Azo + CW1 + PW5	90.5	28.04	62.5	2.23
SEm±	0.9	-	0.9	0.04
LSD (P=0.05)	2.8	-	2.8	0.11

Azo- *Azotobacter* (IARI inoculant); CW1- *Anabaena sp.*; PW5- *Providencia sp.*; RNPK- Recommended dose of nitrogen (N), phosphorus (P) and potassium (K); RPK- Recommended dose of phosphorus (P) and potassium(K)

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Chapter - 5

DISCUSSION

The results obtained during the course of the present study are discussed in this chapter. An attempt has been made to explain the cause and effect relationship which may be responsible for some of the important observations recorded during the course of field investigation to derive valid conclusions. The salient research findings emanating from the study on **"Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)"** are discussed below:

5.1 Plant growth

Data on several growth parameters of wheat, viz. plant height, tillers m^2, dry matter accumulation, leaf area index and growth indices (crop growth rate, relative growth rate and net assimilation rate) were recorded (Table 4.1 to 4.7). The growth of wheat progressed slowly in the beginning and then increased at a faster rate during tillering to flowering stages, and afterwards it slowed down. Growth parameters, particularly plant height and dry matter production, followed the quadratic growth model. Almost all the growth parameters were influenced significantly by application of bacterial and cyanobacterial strains of PGPR (plant growth promoting rhizobacteria).

In general, the highest values of most of the growth parameters were recorded by application of 75% N + RPK + Azo + CW1 + PW5 at all the growth stages studied. The combined application of 75% N with either two or three species of PGPR, viz. 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments favoured significantly higher values of almost all growth attributes in than 75% N + RPK, absolute control or use of all the three species of PGPR without nitrogen use in wheat. The combined use of two or three PGPR species with 75% N was as good as use of recommended NPK in improving the growth parameters of wheat. However, combined use of two or three PGPR species with 75% N and recommended NPK gave significantly higher values of growth parameters over application of any single PGPR species with 75% N. The highest dry matter was produced with application of 75% N + RPK + Azo + CW1 + PW5 which was 47 % higher over absolute control and 13.5% over 75% N + RPK. Similarly, the highest LAI values were recorded with application of 75% N + RPK +

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Azo + CW1 which were 24% and 23% higher in 60 DAS and 90 DAS over 75% N + RPK respectively.

Overall, application of PGPR was very useful in promoting the growth of wheat crop in the present study. PGPR play several key roles in promoting plant growth. They can fix atmospheric nitrogen and make available to the associated crop. Further, some specific strains of PGPR are capable of solubilizing and mobilizing the fixed phosphorus and make it available for plant growth. Prasanna *et al.* (2014) reported that *Azotobacter, Azospirillum* and *Cyanobacteria* can fix 15-20 kg, 20-30 kg and 25-30 kg N ha^{-1}, respectively, under field conditions. Similarly, phosphate solubilizing bacteria (PSB), a kind of PGPR, can mobilize 20-30 kg P_2O_5 ha^{-1} to plant roots (Prasanna *et al*., 2014). Thus the increased availability nitrogen and phosphorus may have helped the wheat plants to accomplish better growth.

Further, the enhance plant growth may also be correlated with increased root growth as recorded higher for the same treatments. Increased root length may have increased the root access to more water and nutrients, particularly micro nutrients which may have resulted in balanced nutrition of the crop. As a general role, accessibility of plants to water and essential nutrients would influence the height of wheat crop through nodes and internodes. Similar results on effect of PGPR on plant height have also been previously reported. Esmailpour *et al.* (2012) reported that inoculation of wheat with *Azotobacter* increase the plant height by 15%. Zahir *et al.* (1998) also reported have also reported an 8.5% increase in height of corn which were infected by *Azotobacter* and *Pseudomonas.*

Many other studies also suggest enhanced growth of plants with respect to many growth parameters in wheat and some other crops. Mirzaei *et al*., (2010) reported that application of *Azotobacter* and *Azospirillum* bacteria at different levels of nitrogen on sunflower increased plant growth characteristics and decreased nitrogen fertilizer application by 50%. Further, Egamberdiyeba (2007) disclosed that bacterial strains *Pseudomonas alcaligenes* PsP15, *Bacillus polymyxa* BC P 26 and *Mycobacterium phlei* M6P19 had a much stimulatory effect on plant growth of maize in nutrient deficient calcisol soil. Furthermore, Nain *et al*. (2010) concluded that plant parameters like, shoot weight, root weight and total biomass was observed highest with inoculation of *bacterial* strains (PW1 + PW5 + PW7) and *Cyanobacteria* CW1+ CW2 + CW3). Steenhoudt and Vanderleyden (2000) reported that PGPR are capable of production of phytostimulators which directly increase plant growth. In addition to

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fixing atmospheric nitrogen, *Azospirillum* spp. are able to give off phytohormones such as auxins, cytokinins and gibberellins which plays key role in the plan growth promotion.

The main and consequent effect of PGPR on plant growth occurs due to increased dry matter production. The increased dry matter production results in increased leaf area, tiller number and leaf area index, etc. Several previous studies have also reported a positive effect of PGPR on dry matter production in wheat and some other crops . Steenhoudt and Vanderleyden (2000) reported that PGPR are capable of production of phytostimulators which directly increase plant growth. Nain *et al*. (2010) concluded that plant parameters like, shoot weight, root weight and total biomass was observed highest with inoculation of *bacterial* strains (PW1 + PW5 + PW7) and *Cyanobacteria* CW1+ CW2 + CW3). Further, Tilak (1992) showed positive effects of combined inoculation of *Azotobacter* and *Azospirillum* on dry matter of maize and sorghum. Manjunath *et al.* (2010) showed that application of *Providencia* sp. (WRB4) had significantly impacted shoot weight succeeded by combined inoculation of *Anabaena oscillariodes* + *Providencia*. Overall, genetic, plant densities, spacing, and fertilization are the major factors affecting the leaf area of the grown under field conditions (Fageria *et al*., 2006). Van and Hartley (2006) have also reported an increase in 30% leaf area of rice due to inoculation of PGPR over no inoculation.

5.2 Yield Attributes

Yield attributes of wheat were significantly affected by application of different combinations of bacterial and cyanobacterial strains (Table 4.8). Absolute control recorded significantly lower spike length, spike weight, and test weight as compared to control + Azo + CW1 + PW5. There was a significant increase with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, and 75% N + RPK + Azo + CW1 + PW5 treatments over 75% N + RPK with respect to the spike length, spike weight, and test weight whereas they were at par with RNPK treatment. In general, combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar values of different yield attributes, all being at par with recommended dose of NPK (RNPK). There was a significant reduction, in general, in values of different yield attributes if only one species (Azo, CW1 or PW5) or no species (75% N + RPK) of PGPR was combined with 75% N + RPK as compared to all the former treatments.

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Several other studies also confirm the findings of the present investigation. Abd EI- Lattief (2013) reported a significant increase in spike length, number of spikelets/spike, kernel weight /spike, 1000-kernel weight of wheat with inoculation of *Azotobacter* and *Azospirillum*. Further, Biswas *et al.* (2000a) showed that rice inoculation with strain E-11 or IRBG-74 of *rhizobium leguminosarum* bv. *trifolii* increased number of panicles per plot and filled grains panicle^{-1}, further, total number of spikelets plant^{-1} were increased over uninoculated plants. In addition, Peng *et al.* (2006) also reported that *Rhizobial* inoculation increased sink size by increasing either in panicle number or spikelet number per panicle. They also showed that increase in spikelet number per panicle was higher at 90 kg N ha^{-1} over zero N_2 application. Furthermore, Choudhary *et al*. (2010) reported that inoculation of *A. brasilense* and *B. subtilis* resulted in statistically similar number of filled grains/panicle, and both showed significantly higher number of filled grains/panicle over no inoculation in rice.

5.3 Rood Studies

The results of the present study showed that 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 have recorded significantly higher root length, volume and dry weight over the application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK, 75% N + RPK. Further, control + Azo + CW1 + PW5 and absolute control produced significantly lower values over all other treatments with respect to root length, volume and dry weight. The highest root length of 4.08 cm cm^{-3} of soil, root volume of 6.78 mm^3 cm^{-3} of soil and dry weight 0.93 mg cm^{-3} of soil were obtained from application of 75% N + RPK + Azo + CW1 + PW5.

The application of PGPR was quite effective in enhancing the wheat root growth in terms of its length, volume and weight. This effect of improved root growth was more pronounced when two or three species of PGPR were combined with mineral N fertilizer (75% N). Improved root systems, including root hair, are the most common phenotypic phenomena noticed after PGPR inoculation in most crops. Consequently, improved root growth and function lead to improved water and mineral uptake, as suggested in the late 1970s. Improved mineral uptake was a popular explanation for the inoculation effects in the 1980–1990s (Bashan and Holguin, 1997).

The increased root length may have increased the root access to more water and nutrients, particularly of micro nutrients. Eghball *et al*. (1993) reported that root

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morphology is influenced by the quantity of N fertilizer applied. Similar reports on increased root growth of wheat in response to inoculation of PGPR are available by other workers. Khalid *et al.* (2004) showed that inoculation of wheat seedlings with PGPR under gnotobiotic (axenic) conditions increases root elongation (up to 17.3%), root dry weight (up to 13.5%), shoot elongation (up to 17.3%) and shoot dry weight (up to 36.3%) over control. Further, Kaci *et al.* (2005) isolated a strain of *Rhizobium* (KYGT207) from an arid soil in southern Algeria. They reported a significant increase in shoot dry mass (85%), root dry mass (56%), root adhering soil (RAS) dry mass (dm) per root dm (RAS/RT) up to 137% and in RAS aggregate water stability by inoculation of wheat with the *Rhizobium* strain KYGT207.

Furthermore, Egamberdiyeva (2007) reported that the bacterial strains *Pseudomonas alcaligenes PsA15*, *Bacillus polymyxa BC P 26* and *Mycobacterium phlei* M6P19 had a much better stimulatory effect on plant growth and N, P and K uptake of maize in nutrient deficient calcisol soil. Their stimulatory efficiency reduced in relatively rich loamy sand soil where bacterial inoculants stimulated only root growth and N, K uptake of root. Manjunath *et al.* (2010) reported that values for root weight were recorded higher for application of *Providencia* sp. (WRB4). Another study was conducted by Rekha *et al.* (2006) to investigate the efficiency of microbial inoculants after encapsulating in alginate supplemented with humic acid on plant growth. They found the highest increase in root length with CC-pg104 free-cell inoculated plants, followed by plants inoculated with encapsulated CC-pg104. Choudhary *et al.* (2010) reported higher values for root length and dry weight with the inoculation of *Azospirillum brasilense*, which were at par to *Bacillus subtilis* inoculation.

5.4 Grain, straw and biological yields

The grain, straw and biological yields of wheat were influenced significantly by application of PGPR. Data indicated that combined application of three PGPR (Azo + CW1 + PW5) alone increased the grain, straw and biological yields of wheat significantly over the absolute control (no PGPR). For example, the increase in grain yield by the former treatment was 10.3% over the later. Hence, the response of PGPR was significant when three species of PGPR were inoculated together even without NPK fertilizers application. Similarly, the inoculation of PGPR increased biological and straw yields by 10% over absolute control.

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It is evident from the data that the highest grain yield was recorded when 75% N was combined with RPK and all the three species of PGPR tested (75% N + RPK + Azo + CW1 + PW5). The next best treatments, with respect to grain yield, were combinations of 75% N + RPK either with any two species of PGPR, i.e. 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5. Combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar grain yields, all being at par with recommended dose of NPK (RNPK). There was a significant reduction in grain yield if only one species (Azo, CW1 or PW5) or no species (75% N + RPK) of PGPR was combined with 75% N + RPK as compared to all the former treatments. Treatments 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and RNPK gave 22.7, 19.3, 17.9, 17.9 and 18.4% higher grain yield, respectively, over 75% N + RPK.

It is thus clear that a significant grain yield response to PGPR inoculation was achieved in the present study. This response was achieved in both situations of no NPK or NPK application. The grain yield increased more profusely if all the three species of PGPR were combined with 75% N + RPK. Interestingly, the grain yield increase was similar when either two or three species of PGPR were combined with 75% N + RPK. The combined use of either two or three species of PGPR with 75% N + RPK was equally effective in increasing the wheat grain yield as application of recommended dose of fertilizers (RNPK). Thus these biofertilizers could help in saving the nitrogenous fertilizers in wheat production. In addition, application of 75% N + RPK produced significantly lower biological and grain yields over RNPK, however the former was at par with application 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5.

Thus it can be inferred that combined application of either one or two species was able to meet the 25 % of nitrogen requirement of wheat crop. However, application of single species was not effective in meeting the nitrogen requirement. Prasanna *et al*. (2014) reported that *Azotobacter*, *Azospirillum* and *Cyanobacteria* can fix 15-20 kg, 20-30 kg and 25-30 kg N ha^{-1}, respectively, under field conditions. Similarly, phosphate solubilizing bacteria (PSB), a kind of PGPR, can mobilize 20-30 kg P_2O_5 ha^{-1} to plant roots (Prasanna *et al*., 2014). Hence, the addition of N and increase phosphorus availability may have contributed towards increased yield of wheat under PGPR inoculation.

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Further, this may also be correlated with increased root parameters as the values recorded were higher for the same treatments. Increased root length may have increased the root access to more water and nutrients particularly micro nutrients which may have resulted balanced crop nutrition. Further, there positive correlation observed between grain yields most of the yield attributes. These finding may also be due to the synthesis of hormones like IAA, which would have promoted the growth of the wheat.

Integration of PGPR with traditional inorganic fertilizers in the field proved to be effective means to increase the availability of nutrients to plants with simultaneous reduction in diseases incidence of oil seed crop has been reported (Kumar *et al*., 2009). The positive effect of many soil bacteria on plants is mediated by a range of mechanisms including improvement of mineral nutrition, enhancement of plant tolerance to biotic and abiotic stress, modification of root development, as well as suppression of soil-borne diseases (Glick, 1995; Glick *et al*. 1999; Kloepper *et al*.1989).

Reddy (2014) have given the ways by which PGPR promote plant growth. These are: increasing nitrogen fixation in legumes, promoting free-living nitrogen-fixing bacteria, increasing supply of other nutrients, such as phosphorus, sulphur, iron and copper, producing plant hormones, enhancing other beneficial bacteria or fungi, and controlling diseases, nematodes and insect pests.

In reviewing the functional diversity of plant growth promoting rhizobacteria (PGPR), Khan *et al.* (2009) suggested several beneficial roles provided from a variety of bacterial taxa. Besides the greater provision of nutrients via nitrogen fixation and phosphate solubilization, bacteria in the rhizosphere also produce phytohormones that regulate root growth and antibiotics and cyanide, which act as biocontrol agents against phytopathogens (Ahmazadeh and Tehrani, 2009) and soil invertebrates (Devi *et al*., 2007). Furthermore, members of the PGPR can act as helper bacteria (Garbaye, 1994) and promote mycorrhizal development of roots by enhancing the recognition system between host and fungus. In their review, Hayat *et al.* (2010) listed the benefits of rhizosphere bacterial communities as symbiotic N-fixation, non-symbiotic N-fixation, phosphorus solubilization, plant growth promotion by phytohormones and siderophore

production.

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Similar reports on increase of yield of various crops plants in response to inoculation of PGPR are available by other workers. Turan *et al*. (2010) showed that combined PGPR inoculation with the strain of OSU-142 + M-13 + *Azospirillum* sp. 245 have significantly enhanced grain yield of wheat over full doses of nitrogen application. Similarly, Rai and Caur (1998) reported that combined inoculation of *Azotobacter* and *Azospirillum* had positive effects on grain yield, biological yield, and harvest index in various wheat genotypes. In addition, Abd EI- Lattief (2013) showed that application of 75% mineral nitrogen and *Azotobacter* increased grain yield by 12.5% over recommended mineral N application, further application of 75% mineral N and *Azotobacter* resulted in 42.2% harvest index.

Kızılkaya (2008) reported that all *Azotobacter* strains increased the grain and straw yield, whereas the maximum increase was obtained from non-indigenous *Azotobacter* strain *Beijerinck* 1901 by 97% in grain yield and 33% in straw yield in pot experiment under greenhouse condition, in contrast, indigenous *Azotobacter* strains like, TK39, RI48, AND RK49 showed promising performance by 74, 70 and 84% in grain yield and 69, 65 and 92% in straw yield, respectively under field condition. Likewise, Yousefi and Barzegar (2013) reported that application of 100% chemical fertilizer with *Azotobacter* and *Pseudomonas* increased biological yield by 12.9% as compared to 100% chemical fertilizer application alone. Meanwhile, Kumar *et al*. (2000) reported that application of mutant strains of *A. chroococcum* increase in grain yield (12.6%) and straw yield (11.4%) over control and their survival (12–14%) was higher in the rhizosphere as compared to their parent soil isolate.

5.5 Microbial Biomass Carbon

This study showed that soil microbial biomass carbon was significantly influenced with application of bacterial and cyanobacterial stains of PGPR. Combined application of three PGPR species (Azo + CW1 + PW5) alone has significantly increased soil microbial biomass carbon over absolute control. Further, it was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 showed significantly higher microbial biomass carbon over both RNPK and 75% N + RPK treatments. Whereas, there was no significant difference observed for the soil microbial biomass carbon among 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5,

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RNPK and 75% N + RPK treatment. The highest microbial biomass carbon was obtained with application of 75% N + RPK + Azo + CW1 + PW5.

The soil microbial biomass is generally a living part of the soil organic matter. It is primarily involved in nutrient transformation, xenobiotic degradation, as a source and sink of C, N, P, S, and enhancing physiochemical properties of the soil (Angers *et al.*, 1992; Gupta and Germida, 1988). Because of its dynamic character, it has been shown to be a sensitive indicator in soil quality measurement under sustainable cropping systems (Anderson and Domsch, 1989; Karlen *et. al.*, 1997). It has also been used to compare microbial carbon and nitrogen content and nutrient cycling between soils under different systems of management (Franzluebbers *et.al.* 1995; Doran and Smith, 1987; Carter and Rannie, 1982). The rational role in use of microbial and biochemical characteristics as soil quality indicator is their central role in cycling of C and N and their senility to change.

The microbial biomass carbon increased significantly in the present study, especially by addition of two or three species of PGPR along with mineral N fertilizer. Similar reports on increase of microbial biomass carbon in wheat in response to inoculation of PGPR are available by other workers. Nain *et al.* (2009) retorted 0.013% increase in all inoculated treatment over uninoculated control. Microbial activity is governed by availability of nutrients in an environment. Percentage of microbial carbon in total organic carbon of soil is strictly to the capacity of soil to support microbial life. The size of microbial biomass carbon is influenced by different management practices, such as crop rotation, biofertilizer application, organic amendments crop residue management, and green manuring. The supply of readily metabolizable C by organic manure and application of biofertilizers are likely to have been most influential factors contributing to the biomass.

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5.6 Dehydrogenase Enzyme Activity

This study indicated that dehydrogenase activity was significantly influenced with application of bacterial and cyanobacterial stains of PGPR. Combined application of three PGPR species (Azo + CW1 + PW5) alone has significantly increased the dehydrogenase enzyme activity over absolute control. Moreover, it was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 showed significantly higher dehydrogenase enzyme activity over both RNPK and 75% N + RPK treatments.

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Whereas, there was no significant difference observed for dehydrogenase activity amongst 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5, RNPK and 75% N + RPK treatment. The highest dehydrogenase activity was obtained with application of 75% N + RPK + Azo + CW1 + PW5.

The soil dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in soil. The basic idea of using soil enzymes activity as a measure of microbial indicators for soil fertility was introduced and established by Waksman (Waksman, 1992). Measurement of dehydrogenase activity represents immediate metabolic activities of soil microorganism at the time of the test. Soil dehydrogenase activity is an oxidative degradation process .i.e., dehydrogenation of organic matter by transferring hydrogen and electrons from substrate to acceptors. Dehydrogenase enzymes play a significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to accepters.

The application of PGPR was truly effective in increasing the dehydrogenase activity. This effect of dehydrogenase activity was more pronounced when two or three species of PGPR were combined with mineral N fertilizer (75% N). The combined application of either two or three species of PGPR indicated higher dehydrogenase activity over application of recommended of chemical fertilizer. The highest dehydrogenase activity was recorded when 75% N was applied with Azo + CW1 + PW5 (75% N + RPK + Azo + CW1 + PW5). It has shown 37% higher dehydrogenase activity over the application of recommended of chemical fertilizers. Further, inoculation of the three PGPR species alone with no chemical fertilizer were found highly effective over absolute control, indeed, the former increase dehydrogenase activity by 29.3% over the later. Similar reports on increase in soil dehydrogenase activity in response to inoculation of PGPR are available by other workers. Manjunath *et al.* (2010) reported that highest dehydrogenize activities were reported with application of *Providencia* sp. (WRB4) with 2/3 application of recommended N. Further, Nain *et al*. (2010) reported that combined application of bacterial and cyanobacterial strains significantly increased the dehydrogenase activity over control.

5.7 Fluorescein diacetate Hydrolysis

The data indicated significant difference for application of different bacterial and cyanobacterial strains of PGPR. Application of 75% N + RPK + Azo + CW1 and

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75% N + RPK + Azo + PW5 showed significantly highest values for FDA hydrolysis over all the other treatments. Whereas, RNPK treatment showed significantly lower values for FDA hydrolysis than 75% N + RPK + Azo, 75% N + RPK + Azo + CW1 and 75% N + RPK + Azo + PW5, and on the other hand it was at par with 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5, 75% N + RPK + CW1 and 75% N + RPK+ PW5. The highest values for FDA hydrolysis were recorded with application of 75% N + RPK + Azo + PW5.

Hydrolysis of fluorescein diacetate, a colourless compound, to Fluorescein which is coloured (Adam and Duncan, 2001), is also assessment of the contribution of several enzymes viz., non-specific esterases, proteases and lipases, all of which take part in the decomposition of organic matter in soil. Since more than 90% of the energy flow in a soil system passes through microbial decomposers and since predominantly heterotrophic microorganisms occur in soil, FDA hydrolysis is thought to reflect overall soil microbiological activity.

The application of PGPR was absolutely effective in increasing the FDA hydrolysis. FDA hydrolysis was higher when *Azotobacter* was inoculated alone or either with CW1 or CW5 and application of 75% N fertilizer. However, the highest values for FDA hydrolysis were obtained when *Azotobacter* was inoculated with CW5 along with 75% N fertilizer. It gave 66% higher value with respect to FDA hydrolysis as compared to application of recommended dose of chemical fertilizer. Similar reports on increase in FDA hydrolysis on various crops in response to PGPR inoculation are available by other workers. Nain *et al*. (2010) observed highest values for FDA hydrolysis and with application of two bacterial strains (PW_1 + PW_7) and one *Cyanobacteria* strain (CW_2). Further, Rana *et al*. (2012) reported a significant enhancement in FDA hydrolase with application of (AW5 + AW7) treatment compared to all the treatments at the mid and harvest stages of the wheat crop.

5.8 Concentration and Uptake of Nitrogen (N) in Grain and Straw and Protein Content in Grain

Concentration and uptake of N in grain and straw, and protein content in grain were significantly influenced with the application of bacterial and cyanobacterial strains of PGPR. The results showed that N concentration and uptake in grain and straw, and protein content in grain were significantly lower in absolute than inoculation of three species of PGPR (Azo + CW1 + PW5) alone. Further, 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75%

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N + RPK + Azo + CW1 + PW5 treatments significantly increased N concentration and uptake in grain and straw, and protein content in grain over 75% N + RPK, whereas the former treatments were at par with RNPK treatment. Application of 75% N + RPK + Azo, 75% N + RPK + CW1, 75% N + RPK+ PW5 did not significantly increase N concentration and uptake in grain and straw, and protein content in grain over RNPK, however, they were at par with application of 75% N + RPK. The highest values for N concentration and uptake in grain and straw, and protein content in grain were obtained with application of 75% N + RPK + Azo + CW1 + PW5.

The highest N concentration in grain sand straw and protein content in grain was obtained from combined application of either two or three species of PGPR. Nitrogen is the most important nutrient for plant growth and productivity. In addition to enhancement of plant growth and yield, PGPR are directly involved in increased concentration and uptake of nitrogen and synthesis of phytohormones. This may be correlated with their ability to fulfill the N requirement of the wheat crop and the application of single species may have failed to meet nitrogen requirement. Further, this may also be correspondent with increased root parameters as recorded higher for the same treatments. Increased root length may have increased the root access to more water and nutrients particularly micro nutrients which have resulted in balanced nutrition of the wheat crop.

The application of PGPR was quite effective in increasing the protein content of wheat. It was found that combined inoculation of either two or three species of PGPR with mineral N fertilizer (75% N) gave higher protein in wheat. They increased protein content of wheat by 34.5% over the application of 75% of N alone. Further, the protein content of the former treatments were also found higher over the single inoculation of any one of the species with mineral N fertilizer (75% N). In addition, Hassanpour *et al.*, reported highest amount for protein percentage (12.4%) with combined inoculation of mycorrhizae and *Azotobacter* in wheat. Further Roesti *et al.* (2006) reported significantly higher protein content in the treated plants grain over the control plants and maximum values were achieved when PGPR were co-inoculated with the AMF in rain-fed wheat field.

The application of PGPR was highly effective in increasing the N concentration in grain and straw, and total N uptake. The data revealed that combined inoculation of either two or three species of PGPR with mineral N fertilizer (75% N), increased the nitrogen concentration both in grain and straw, and total N uptake.

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Highest N concentration in grain was obtained with inoculation of all of the three PGPR species along with 75% N application. The N concentration and total N uptake was the highest in combined inoculation of all the three PGPR species along with 75% N application. The application of (75% N + RPK + Azo + CW1 + PW5) was found to increase N concentration in straw by 37% and total N uptake by wheat 60% over the application of 75% N + RPK).

Similar reports on increase in concentration and uptake in grain and straw and protein content in grain of various crops plants in response to inoculation of PGPR are available by other workers. Turan *et al.* (2010) showed that highest N concentration in leaf, grain, and straw were obtained from mixed inoculation with the OSU-142 + M-13 + *Azospirillum* sp.245 +40 kg N ha^{-1}, which increased N concentration of leaf, grain and straw of wheat crop by 52.6%, 83.4%, and 83.0%, respectively, over the control treatment. Further, Das and Saha (2003) reported that inoculation of soil with *Azospirillum* in partial application of nitrogen fertilizer showed highest stimulation of these microaerophilic N_2-fixing bacteria in the rhizosphere. And Choudhary (2008) also showed significant effect of PGPR on N concentration in grain and straw of rice. Kapulnik *et al.* (1981) reported that inoculation significantly increased the total N concentration in shoots and grains of inoculated plants.

5.9 Economic analysis of wheat cultivation

The application of bacterial and cyanobacterial strains significantly influenced gross return, net return, and benefit cost ratio (Table 4.17). The results indicated that there was no significant difference with application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 and RNPK in gross return, net return and benefit cost ratio, but they were significantly higher from 75% N + RPK. However, highest gross return (₹ 90,500 ha^{-1}), net return (₹ 62,500 ha^{-1}) and benefit cost ratio (2.17) were obtained by application of 75% N + RPK + Azo + CW1 + PW5. The lowest gross return (₹ 56,200 ha^{-1}) and net return (₹ 32,000 ha^{-1}) and benefit cost ratio (1.32) were achieved with absolute control.

Overall, economic returns were higher when two or three strains of PGPR were combined with 75% N + full PK fertilizers. Further, the grain and straw yields were almost similar with combined use of two or three strains of PGPR + 75% N + full PK fertilizers and full dose of NPK fertilizer treatment. Therefore, it can be inferred that

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use of two or three PGPR strains with 75% N +PK fertilizers saved 25 % nitrogen in wheat cultivation. These PGPR strains are much cheaper than cost of N fertilizer. Since the cost of cultivation was reduced when two or three PGPR strains were combined with 75% N +PK fertilizers than 100% NPK fertilizer, hence, the former treatments gave better returns and benefit:cost ratio.

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SUMMARY & CONCLUSION

The experiment was carried out on a sandy clay loam soil at "main block 14C" research farm of Indian Agricultural Research Institute, New Delhi 110 012, India during the Rabi season of 2014-2015. The experiment was entitled **"Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)"**. The objective of the study was to find out the effect of plant growth promoting rhizobacteria (PGPR) on the growth, and productivity of wheat, further to see the effect of PGPR on the NPK uptake and grain quality of wheat and finally to work out the economics of PGPR inoculation in wheat. The experiment was laid out in randomize complete block design with three replication under irrigated condition. The experimental plots received six irrigations and two hand weedings and applied NPK fertilizers based on the treatment combination.

The most important findings of the experiment are summarized as bellow.

1. Effect of plan growth promoting rhizobacteria were recorded significant for most of the parameters under study.
2. The combined application of either two or three bacterial or cyanobacterial species were able to fix sufficient atmospheric nitrogen to meet the 25% N requirement of the wheat crop which reduced over the recommended N application, whereas application of single species failed to do so.
3. Application PGPR did not influence the growth parameters viz., plant height, number of tillers m^{-2} and dry matter accumulation in 30 days after sowing.
4. Combined application 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 significantly increased growth parameters viz., plant height, number of tillers m^{-2} and dry matter accumulation over 75% N + RPK.
5. The highest leaf area were obtained from application of 75% N + RPK + Azo + CW1.
6. Yield attributing characters viz., spike length, spike weight, grain weight per spike, number of grains per spike and test weight were significantly influenced with application of bacterial and cyanobacterial strains. Application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 +

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PW5, and 75% N + RPK + Azo + CW1 + PW5 recorded significantly higher values for spike length, spike weight, grain weight per spike, number of grains per spike and test weight treatments over 75% N + RPK whereas they were at par with RNPK treatment.

7. The highest crop growth rate and relative growth rate were obtained from application of 75% N + RPK + Azo + CW1 + PW5.
8. The results showed that 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 have recorded significantly higher root length, root volume and root dry weight over all other treatments. However the highest root length 4.08 cm per cm^{-3} of soil, root volume 6.78 mm^{-3} per cm^{-3} of soil and dry weight 0.93 mg per cm^{-3} of soil were obtained from application of 75% N + RPK + Azo + CW1 + PW5 which were 4%, 30%, and 52% higher from application RNPK respectively.
9. Combined application of bacterial and cyanobacterial inoculants in 75% N + RPK + *Azo* + CW1, 75% N + RPK + *Azo* + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + *Azo* + CW1 + PW5 treatments produced significantly higher grain and straw yield over 75% N + RPK but they were at par with RNPK treatment.
10. Highest values of gain yield were obtain from application 75% N + RPK + *Azo* + CW1 + PW5 which was 77% and 23% higher over absolute control and fertilizer control (75% N + RPK) respectively, whereas application of 75% N + RPK + *Azo* + PW5 produced highest straw yield which was 35.5% and 13% higher over absolute control and fertilizer control (75% N + RPK) respectively.
11. It was observed that application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 showed significantly higher values over both RNPK and 75% N + RPK treatments with respect to the soil microbial biomass carbon whereas soil organic carbon was not influenced with application of PGPR.
12. The highest values for dehydrogenase activity and soil chlorophyll were obtained from application of 75% N + RPK + Azo + CW1 + PW5.
13. The highest values for FDA hydrolysis was recorded from application of 75% N + RPK + Azo + PW5. The treatment showed 66% and 33 % higher values over absolute control and 75% N + RPK respectively.

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14. The highest gross return (₹ 90,500 ha^{-1}), net return (₹ 62,500 ha^{-1}) and benefit cost ratio (2.17) was obtained from application of 75% N + RPK + Azo + CW1 + PW5.
15. Application of 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5, 75% N + RPK + Azo + CW1 + PW5 treatments showed significantly higher values for N concentration and uptake in grain and straw and protein content in grain over 75% N + RPK, whereas they were at par RNPK treatment
16. The highest P concentration in grain and straw and uptake was recorded in application of 75% N + RPK + Azo + CW1 + PW5.
17. The highest agronomic efficiency was obtain from application of 75% N + RPK + Azo + CW1 + PW5 treatment

Conclusion

Reduction in soil fertility, low fertilizer use efficiency, increasing environmental pollution and are the primary concern to agriculture in tem of crop productivity. Biofertilizers are appropriate and environmental friendly supplement to the chemical fertilizers. This study clearly showed the potential positive influence of PGPR on productivity and nutrient and nutrient use of efficiency of wheat. Different combinations of bacterial strain *Providencia* sp. PW5, *Azotobacter* (IARI inoculant), and cyanobacterial strain *Anabaena laxa* CW1 have significantly increased growth and yield attributing characters of wheat which resulted in significant increase both in grain and straw yield. The combined application of either two or three species of bacterial inoculants can saved 37.5 kg N/ha without significantly reducing yield. This reduction in the N application will reduce rate of the N losses and its harmful effects both on human and the environment and reduce the cost of fertilizer supply. Further the combined application of these PGPR inoculants resulted in significant increase in root growth. This may increase root access to more nutrients and water absorption. The study shows that application of biofertilizers in long term can enhance soil biological properties.

Future Scope of Research

Experiments on plant growth promoting rhizobacteria should be conducted in low in put agriculture systems (farmer field) to evaluate their potential characters.

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Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)

ABSTRACT

Wheat (*Triticum aestivum* L.) was grown on 29.4 million hectares with production of 92.3 million tonnes and productivity of 3.1 tonnes per hectare in India during 2012-13. The wheat productivity is still much low in India than in the world. However, it can be increased by growing suitable variety, proper water management, weed control, insect-pests and disease management, and by judicious nutrient management. Among all the plant nutrients, nitrogen is needed by wheat in largest amounts. Nitrogen is supplied mainly by chemical fertilizers which are expensive and pollute the environment. The alternative way could be the use of plant growth promoting rhizobacteria (PGPR) to substitute the requirement of nitrogenous fertilizer partially. The PGPR have been found effective in fixing atmospheric nitrogen, mobilizing phosphorus to plant roots, enhancing root and shoot growth of crops by producing certain hormones in soil and suppressing the plant disease. Therefore, keeping the above facts in view a field experiment entitled "Effect of plant growth promoting rhizobacteria on productivity and nutrient use efficiency of wheat (*Triticum aestivum* L.)" was carried out at the Research Farm of ICAR-Indian Agricultural Research Institute, New Delhi, India, during *rabi* season of 2014-15. The objectives of the field study were: (i) to find out the effect of plant growth promoting rhizobacteria (PGPR) on root and shoot growth, and productivity of wheat, (ii) to estimate the NPK uptake and grain quality of wheat under varied treatments of PGPR inoculation, and (iii) to work out the economics of PGPR inoculation in wheat.

The experiment was laid out in randomized block design with three replications. Treatments (11) were: absolute control, control + *Azotobacter* (IARI Inoculant) + CW1 (*Anabaena sp*.) + PW5 (*Providencia sp*.), recommended dose of NPK (RDF), 75% N + Full dose PK,75% N + Full dose PK + *Azotobacter* (IARI Inoculant), 75% N + Full dose PK + CW1 (*Anabaena* sp.), 75% N + Full dose PK + PW5 (*Providencia* sp.), 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) + CW1 (*Anabaena* sp.), 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) + PW5 (*Providencia sp*.), 75% N + Full dose PK + CW1 (*Anabaena* sp.) + PW5 (*Providencia* sp.), and 75% N + Full dose PK + *Azotobacter* (IARI Inoculant) CW1 (*Anabaena* sp.)

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+ PW5 (*Providencia* sp.). Wheat variety 'HD 2967' was sown on 18th November 2014 at a spacing of 22.5 cm. The crop was harvested on 17th April 2015. All the necessary observations were recorded during the study.

The results showed that the highest values of most of the growth parameters (viz., plant height, number of tillers m^{-2} and dry matter accumulation) were recorded by application of 75% N + RPK + Azo + CW1 + PW5 at all the growth stages. The combined application of 75% N with either two or three species of PGPR, viz. 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5 and 75% N + RPK + Azo + CW1 + PW5 treatments favoured significantly higher values of almost all growth attributes than 75% N + RPK. In general, combinations of either two or three species of PGPR with 75% N + RPK produced statistically similar values of different yield attributes, all being at par with recommended dose of NPK (RNPK). The grain, straw and biological yields of wheat were influenced significantly by application of PGPR. Data indicated that combined application of three PGPR (Azo + CW1 + PW5) alone increased the grain, straw and biological yields of wheat significantly over the absolute control (no PGPR). The highest grain yield was recorded when 75% N was combined with RPK and all the three species of PGPR tested (75% N + RPK + Azo + CW1 + PW5). The next best treatments, with respect to grain yield, were combinations of 75% N + RPK either with any two species of PGPR, i.e. 75% N + RPK + Azo + CW1, 75% N + RPK + Azo + PW5, 75% N + RPK + CW1 + PW5. The highest agronomic efficiency of nitrogen was obtained with application of 75% N + RPK + Azo + CW1 + PW5 treatment. The highest gross return (₹ 90,500 ha-1), net return (₹ 62,500 ha-1) and benefit cost ratio (2.17) was also obtained by application of 75% N + RPK + Azo + CW1 + PW5.

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पादप वृद्धि प्रोत्साहक राइज़ोबैक्टीरिया का गेहूं (*ट्रिटीकम एस्टिवम* एल.) की उत्पादकता एवं पोषक तत्व उपयोग दक्षता पर प्रभाव

सारांश

वर्ष 2012-13 के अंतर्गत भारत में गेहूं (*ट्रिटीकम एस्टिवम* एल.) 29.4 मिलियन हैक्टेयर क्षेत्र में उगाया गया जिसका कुल उत्पादन 92.3 मिलियन टन और उत्पादकता 3.1 टन प्रति हैक्टेयर थी। विश्व की तुलना में भारत में गेहूं की उत्पादकता आज भी काफी कम है। हालांकि इसकी उत्पादकता को उपयुक्त किस्म को उगाकर, उचित जल प्रबंध, खरपतवार नियंत्रण, कीट-पीड़क एवं रोग प्रबंधन, और समुचित पोषक तत्व प्रबंधन करके बढ़ाया जा सकता है। सभी पोषक तत्वों में गेहूं को नाइट्रोजन की सर्वाधिक आवश्यकता होती है। नाइट्रोजन की पूर्ति मुख्य रूप से रासायनिक उर्वरक द्वारा की जाती है जो महंगे होते हैं और पर्यावरण प्रदूषण भी बढ़ाते हैं। नाइट्रोजन उर्वरक की आंशिक रूप से आवश्यकतापूर्ति के लिए वृद्धि प्रोत्साहक राइज़ोबैक्टीरिया (पी जी पी आर) के प्रयोग से स्थानापन्न करना एक वैकल्पिक तरीका हो सकता है। पी जी पी आर को वायुमंडलीय नाइट्रोजन स्थिरीकरण करने में, फास्फोरस को पादप जड़ों तक पहुंचाने में, कुछ विशिष्ट हार्मोन्स का स्राव करके पौधों के प्ररोह एवं जड़ों की वृद्धि करने में, और पादप रोगों के दमन में प्रभावकारी पाया गया है। अतः उपरोक्त बिन्दुओं को ध्यान में रखते हुए भा.कृ.अनु.प.-भारतीय कृषि अनुसंधान संस्थान, नई दिल्ली के अनुसंधान प्रक्षेत्र में *रबी* (नवम्बर – अप्रैल) वर्ष 2014-15 में एक अनुसंधान शीर्षक "पादप वृद्धि प्रोत्साहक राइज़ोबैक्टीरिया का गेहूं (*ट्रिटीकम एस्टिवम* एल.) की उत्पादकता एवं पोषक तत्व उपयोग दक्षता पर प्रभाव" पर किया गया। इसके उद्देश्य थे: (i) वृद्धि प्रोत्साहक राइज़ोबैक्टीरिया (पी जी पी आर) के गेहूं की जड़ एवं प्ररोह वृद्धि, और उत्पादकता पर प्रभाव की जांच करना, (ii) पी जी पी आर टीके के विभिन्न उपचारों द्वारा गेहूं की फसल में नाइट्रोजन अवशोषण एवं गुणवत्ता का आकलन करना, और (iii) गेहूं में पी जी पी आर उपचारों की आर्थिकी पर कार्य।

यह प्रयोग यादृच्छिक भाग आकार में तीन पुनरावृत्ति के साथ किया गया। उपचार (11) थे: पूर्णतया नियंत्रित, नियंत्रित + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.), एन पी के की संस्तुत मात्रा (आर डी एफ), 75% ना. + पी के की सम्पूर्ण मात्रा, 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका), 75% ना. + पी के की सम्पूर्ण मात्रा + सी डब्ल्यू 1 (*एनाबिना* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.) और 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.). गेहूं की 'एच डी 2967' प्रजाति की बुवाई 14 नवंबर 2014 को 22.5 सें.मी. दूरी पर की गई। फसल की कटाई 17 अप्रैल 2015 को की गई थी। अध्यन के दौरान सभी आवश्यक आंकड़े एकत्रित किए गए थे।

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परिणामों से ज्ञात हुआ कि अधिकतर वृद्धि मापदंड (जैसे पादप ऊंचाई, प्रति वर्ग मीटर कल्लों की संख्या एवं शुष्क भार संचयन) के मान सभी वृद्धि अवस्थाओं पर उपचार 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.) के प्रयोग से सर्वाधिक पाए गए। 75% ना. एवं पी जी पी आर की दो अथवा तीन प्रजातियों के संयुक्त प्रयोग जैसे -75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.), 75% ना. + पी के की सम्पूर्ण मात्रा + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.) और 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* (आई ए आर आई टीका) + सी डब्ल्यू 1 (*एनाबिना* स्प.) + पी डब्ल्यू 5 (*प्रोवीडेंसिया* स्प.) द्वारा 75% ना. + पी के की सम्पूर्ण मात्रा उपचार की तुलना में वृद्धि घटकों के मान में सार्थक एवं अनुकूल मान प्राप्त किए गए। सामान्यतः 75% ना.+ पी के की सम्पूर्ण मात्रा एवं पी जी पी आर की दो अथवा तीन प्रजातियों के संयुक्त प्रयोग करने से विभिन्न उपज घटकों के मान एन पी के की संस्तुत मात्रा (आर डी एफ) के समान प्राप्त हुए। पी जी पी आर के प्रयोग का गेहूं की जैविक, दाना एवं भूसा उपज पर सार्थक प्रभाव पड़ा। आंकड़ों से प्रकट होता है कि पी जी पी आर की केवल तीन प्रजातियों के संयुक्त प्रयोग (*एजोटोबैक्टर* + सी डब्ल्यू 1 + पी डब्ल्यू 5) से पूर्णतया नियंत्रित (पी जी पी आर नही) उपचार की तुलना में सार्थकता के आधार पर गेहूं की अधिक जैविक, दाना एवं भूसा उपज प्राप्त हुई। दाने की अधिकतम उपज तब दर्ज की गई जब 75% ना. का पी के की सम्पूर्ण मात्रा एवं पी जी पी आर की सभी तीनों प्रजातियों (75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* + सी डब्ल्यू 1 + पी डब्ल्यू 5) के साथ संयुक्त प्रयोग किया गया। दाने की उपज के दृष्टिकोण से अगले श्रेष्ठ उपचार थे: 75% ना.+ पी के की सम्पूर्ण मात्रा का पी जी पी आर की दो प्रजातियों के संयुक्त प्रयोग, जैसे-75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* + सी डब्ल्यू 1, 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* + पी डब्ल्यू 5, 75% ना. + पी के की सम्पूर्ण मात्रा + सी डब्ल्यू 1 + पी डब्ल्यू 5। गेहूं में नाइट्रोजन की उच्चतम सस्यीय दक्षता 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* + सी डब्ल्यू 1 + पी डब्ल्यू 5 उपचार से प्राप्त की गई। उच्चतम कुल लाभ (रु. 90,500/ है.), शुद्ध लाभ (रु. 62,500/ है.), एवं लाभ: कीमत अनुपात (2.17) भी उपचार 75% ना. + पी के की सम्पूर्ण मात्रा + *एजोटोबैक्टर* + सी डब्ल्यू 1 + पी डब्ल्यू 5 से प्राप्त हुए।

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Chapter – 7

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ANNEXURE- I

Meteorological data during the crop season

Date	Rainfall (mm)	Temperature (°C)		Evaporation (mm)	RH-I (%)	RH-II (%)	BSS (hrs)
		T Max.	T Mini.				
12-11-14	0	27.5	9.2	3.5	88	35	6.6
13-11-14	0	26.8	8.6	3.5	81	38	3.1
14-11-14	0	26.2	7	4	87	33	5.4
15-11-14	0	27	8.2	4	74	37	5.2
16-11-14	0	27	7.5	3.4	86	38	7.2
17-11-14	0	27	7.7	4	89	45	7
18-11-14	0	27.4	7.5	4.2	80	42	7.8
19-11-14	0	26.8	6.8	5	90	46	7.3
20-11-14	0	27.8	8.2	3.8	87	48	6.8
21-11-14	0	28	8	3.3	92	53	6.8
22-11-14	0	27	7	3.4	87	39	5.9
23-11-14	0	27	7.4	3.6	84	40	6.4
24-11-14	0	27	6.4	3.8	83	33	6.8
25-11-14	0	25.5	6	3	91	33	6.2
26-11-14	0	26.2	7.5	4.2	79	35	7.2
27-11-14	0	26.4	8	3.9	87	32	6.2
28-11-14	0	27.2	10.2	3.4	82	35	6.4
29-11-14	0	28.2	11.6	4	80	39	5.4
30-11-14	0	29	11.2	4	98	31	5.5
01-12-14	0	29.5	11.3	4	79	34	7.3
02-12-14	0	29	13.2	3.5	80	43	7.8
03-12-14	0	27.4	13.2	4	79	46	7.7
04-12-14	0	27.8	9.9	4	90	45	7.6
05-12-14	0	27.5	7.8	3.5	95	45	7.9
06-12-14	0	27.4	7.7	2.4	97	51	7.8
07-12-14	0	26.4	10.9	3.3	81	42	7.4
08-12-14	0	26.7	7.2	4.3	94	49	8.2
09-12-14	0	25.5	6.4	3	97	40	7.1
10-12-14	0	26.5	6.2	3	94	23	6.2
11-12-14	0	23.8	3.2	2.2	90	49	7.2
12-12-14	0	22	2.6	2.9	87	39	6.9
13-12-14	0	22	10	2.4	87	64	5.2
14-12-14	0	20	13.4	3.7	98	98	0
15-12-14	26.4	16	10.8	3.1	97	75	0
16-12-14	0	18.7	7.8	2.1	97	75	3.3
17-12-14	0	19	8.4	2	97	59	4.9
18-12-14	0	18.5	6.7	1.7	97	76	4.4
19-12-14	0	16	5.5	2	97	78	2.5
20-12-14	0	15.2	6.2	1.5	97	68	0
21-12-14	0	15.4	5.9	1.8	97	70	4.5
22-12-14	0	13	1.9	1.4	97	64	0
23-12-14	0	15	5.2	1.3	100	69	0

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24-12-14	0	14	3	1.3	97	84	2.1
25-12-14	0	13.6	5	1	97	70	0
26-12-14	0	13.6	5.3	1.8	97	52	2.1
27-12-14	0	16.2	2.1	1.4	100	58	1.3
28-12-14	0	18.2	-0.9	0.9	100	79	4.9
29-12-14	0	16.7	3.4	1.5	100	53	2.2
30-12-14	0	19.4	2.2	2	97	62	5.6
31-12-14	0	18.5	7	2	95	70	4.1
01-01-15	0	17.2	7.2	2	97	67	1
02-01-15	5	21	9.8	1	98	100	0
03-01-15	13.6	16	12.9	1.4	100	92	0
04-01-15	0	17	11.5	1	93	73	0
05-01-15	0	18	7	1.5	94	68	3.3
06-01-15	0	20	8	3	97	76	6.4
07-01-15	0	16	4.3	2.9	97	83	2.2
08-01-15	0	12.5	5.8	1.6	94	86	0
09-01-15	0	13	5.6	1	94	62	0
10-01-15	0	14.4	6.4	1.7	98	73	0
11-01-15	0	17.1	4.1	2.9	97	78	4.7
12-01-15	0	15.4	3.3	1.6	100	68	0
13-01-15	0	15.4	1.6	3	98	61	0
14-01-15	1.4	17.6	9.9	3	100	86	4.7
15-01-15	0.4	16.2	3.4	2	99	69	0
16-01-15	0	18	3.9	2	97	63	2.2
17-01-15	0	19	4.9	1.6	97	68	5.2
18-01-15	0	20	2.6	1.8	97	48	4.4
19-01-15	0	20	7	2	97	54	4.9
20-01-15	0	19.5	6.6	1.4	95	68	3.5
21-01-15	0	17	8.5	1	92	58	0
22-01-15	4	17	10	1.8	98	88	0
23-01-15	8	13	10.6	1.2	98	73	0
24-01-15	0	18.4	9.6	2	97	86	1.6
25-01-15	0	14.6	7.5	1.3	97	63	0
26-01-15	2.8	16	9.8	1.7	95	79	4.2
27-01-15	0.6	16.4	8.8	1.1	95	72	0
28-01-15	0	17	7.2	1.2	92	53	1.6
29-01-15	0	17	2.5	1.8	97	43	6.1
30-01-15	0	17.2	4.5	2	94	40	7.2
31-01-15	0	18.4	6.5	2.2	83	37	8
01-02-15	0	20.2	4.5	2.4	91	36	7.3
02-02-15	0	23	10.5	2.8	88	52	3.5
03-02-15	0	22	13.4	2.4	87	73	4.7
04-02-15	0	22	11	2.3	98	77	0.7
05-02-15	0	19	8.2	2.2	95	36	2.7
06-02-15	0	22	5	2.7	97	43	8
07-02-15	0	23	5	1.7	97	40	7.6

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08-02-15	0	24	6.5	2.7	92	38	7.2
09-02-15	0	23.5	10	2.4	88	39	7.4
10-02-15	0	24.5	6	3	97	42	8.4
11-02-15	0	22.5	6	2.5	91	29	8.1
12-02-15	0	23.5	6	3	97	53	6.2
13-02-15	0	22	7.6	3	94	42	6
14-02-15	0	24.4	8.4	3.4	90	43	8.4
15-02-15	0	26.5	8.4	3.7	92	43	7.4
16-02-15	0	25.5	12.8	3	98	59	4.3
17-02-15	0	25.5	15.2	2.6	90	56	2.7
18-02-15	0	25.5	12	3.4	98	53	2.4
19-02-15	0	27.1	15.6	4.3	86	63	1.3
20-02-15	0.02	27.6	18.3	4.5	89	65	1.8
21-02-15	0	27.7	13.4	3.9	93	54	4.1
22-02-15	0	27	15.2	5.6	90	46	6.9
23-02-15	0	27.2	14.2	5.9	98	59	7.8
24-02-15	0	27.4	12.6	4.6	98	59	2.1
25-02-15	0	28	16.5	2.2	85	64	0
26-02-15	0	27	13.4	2	85	32	0.7
27-02-15	0	26	8.9	4.7	87	27	7.7
28-02-15	0	26	12.5	3.2	82	30	7
01-03-15	14.4	27.2	14.4	3.4	98	96	6
02-03-15	79.4	18	13	1.8	96	82	0
03-03-15	41.6	21	10.5	1.6	98	69	1.5
04-03-15	0	21	9.2	2.2	98	50	3.5
05-03-15	0	22.4	7.6	4	97	39	8.3
06-03-15	0	25.2	6	3.3	94	46	8.8
07-03-15	0	26	10.2	3.8	89	48	7.4
08-03-15	45	26	12.4	3.9	95	59	5.5
09-03-15	0	23.6	11.8	3	86	44	3.4
10-03-15	0	22.5	9	5	90	58	8.5
11-03-15	0	23.2	10.2	4.6	95	56	8.5
12-03-15	0	25	9.5	4.5	95	46	7.2
13-03-15	0	27	12.2	4	98	42	7.8
14-03-15	0	27.4	13.2	5.1	96	53	1.9
15-03-15	0	26	15.7	4	81	86	1.3
16-03-15	0.4	20.6	13.4	3.3	98	53	0
17-03-15	0	25.2	12.9	2.5	89	51	7.6
18-03-15	0	27	13.4	4	91	49	8.7
19-03-15	0	25.2	11.9	4.3	91	39	9.4
20-03-15	0	27	11.5	5.4	93	37	9.2
21-03-15	0	29	13.5	5.6	98	42	9.2
22-03-15	0	31	13	6	85	42	9.4
23-03-15	0	31	13.6	6.6	85	43	9.8
24-03-15	0	31.8	15.2	7.8	84	35	10.3
25-03-15	0	33.2	15.9	6.5	92	37	10

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26-03-15	0	34	17.8	5	91	34	7.8
27-03-15	0	35.2	20.2	4.6	74	36	6.7
28-03-15	0	34.5	14.9	4.9	88	36	9.9
29-03-15	0	34.7	18.2	5.4	71	50	8.9
30-03-15	21	34.2	18.9	5.1	87	69	0
31-03-15	0	27.5	18.2	3	92	54	6.4
01-04-15	0	30	16.7	3.9	98	45	6.3
02-04-15	0	32.2	19.2	5.4	81	44	7.7
03-04-15	14.2	33.4	18.4	4.9	98	63	6.9
04-04-15	31.2	29.4	16.5	4	98	68	4.5
05-04-15	0	27.7	14.4	4.6	92	52	4.7
06-04-15	0	29	15.6	3.9	91	37	7.9
07-04-15	0	31	18.2	4.8	85	51	7.6
08-04-15	0.4	28.5	16.5	4.2	85	37	6.2
09-04-15	0	31.5	17	3.5	77	42	9.1
10-04-15	0	32	17.4	5.1	79	38	9.8
11-04-15	0	34.4	17.2	6.6	72	33	9.6
12-04-15	6	35.6	17	6	73	66	6.2
13-04-15	0	27.5	17	3.5	91	49	0
14-04-15	0	30.8	19.4	4.4	72	47	6
15-04-15	0	31.5	19	6.2	77	46	9.9
16-04-15	0	33.2	19.2	6.4	78	55	8.7
17-04-15	0	31	18.9	6	84	57	3.4
18-04-15	0	35.2	19.4	6.6	83	37	7.9
19-04-15	0	38.2	19.4	8	75	32	8.3
20-04-15	0	40.5	24.6	7.2	52	35	8.5
21-04-15	0	38.7	24.5	7.4	70	25	5.5
22-04-15	0	39	17.4	8.4	66	25	7.2

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ANNEXURE-II

Estimation cost of cultivation of wheat (2014-15)

Particular	No./ quantity/ha	Unit cost (Rs)	Total Cost
Discing	6 tractor	200	1200
Ploughing by cultivator and planking	6 tractor hr	200	1200
Bunding	4 man-days	250	1000
Seed	100 kg	25/ kg	2500
Sowing and planking	4 tractor hr	200	800
Sub total			6700
Fertilizers (kg)			
	N: 150 kg	11.54/ kg	1731
	P_2O_5 : 60 kg	21.25/ kg	1275
	K_2O : 40 kg	7.43/ kg	297
Seed treatment (biofertilizer)	741 g	20 /200 g pocket	74
Fertilizer application			
Basal (1/2 N + full PK+ biofertilizer)	2 man-day	250	500
Top dressing (1splits of N)	1 man-day	250	250
Sub total			4127
Weed management			
Hand weeding (First)	10 man-day	250	2500
Hand weeding (Second)	12 man-day	250	3000
Sub total			5500
Irrigation			
Irrigation (cost of water)	5 irrigations	400	2000
Application cost	5 man-days	250	1250
Sub total			3250
Harvesting			
Harvesting	10 man-days	250	2500
Threshing / cleaning bagging	15 man-days	20	3750
Sub total			6250
Rental value of land	1 ha (5 months)	2500	2500
Total			28327

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ANNEXURE-III

Estimation of treatment wise cost of cultivation of wheat

Treatment	Cost of PGPR	Cost of N	Cost of $P_2O_5 + K_2O$	Cost of fertilizer application	Total fertilizer costs	Common costs	Total costs of cultivation
Absolute control	0	0	0	0	0	24200	24200
Control + *Azotobacter* + CW1 + PW5	222	0	0	250	472	24200	24672
Recommended dose of NPK	0	1731	1572	750	4053	24200	28253
75% N + Full dose PK	0	1298	1572	500	3370	24200	27570
75% N + Full dose PK + *Azotobacter*	74	1298	1572	750	3694	24200	27894
75% N + Full dose PK + CW1	74	1298	1572	750	3694	24200	27894
75% N + Full dose PK + PW5	74	1298	1572	750	3694	24200	27894
75% N + Full dose PK + *Azotobacter* + CW1	148	1298	1572	750	3768	24200	27968
75% N + Full dose PK + *Azotobacter* + PW5	148	1298	1572	750	3768	24200	27968
75% N + Full dose PK + CW1 + PW5	148	1298	1572	750	3768	24200	27968
75% N + Full dose PK + *Azotobacter* + CW1 + PW5	222	1298	1572	750	3842	24200	28042

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