

Chapter 5

Role of Bio-fertilizers in Crop Improvement

Majeed-ul-Hassan Chesti, Tabasum N. Qadri, Asiya Hamid, Javed Qadri, Mohamed Mahgoub Azooz and Parvaiz Ahmad

Abstract Bio-fertilizers are cost effective, ecofriendly and renewable source of plant nutrients to supplement chemical fertilizers in sustainable agricultural system. Bio-fertilizers are preparations containing living cells of efficient nitrogen fixing, P-solubilizing/mobilizing or cellulose decomposing microorganisms, which when applied to seed or inoculated into the soil enhance availability of nutrients to plants either working symbiotically/asymbiotically or through solubiization of soil nutrients such as phosphorus or decomposition of complex materials. Bio-fertilizers are gaining impetus due to the growing emphasis on maintenance of soil health, curtail the environmental pollution and cut down on the use of chemicals in agriculture. Bio-fertilizers are also ideal input for reducing the cost of cultivation and for practicing organic farming. In the present context of very high cost of chemical fertilizers, the bio-fertilizers assume special significance.

M.-ul-H. Chesti (✉) · J. Qadri
Regional Agricultural Research Station, SKUAST-J, Tandwal, Rajouri 185131,
Jammu and Kashmir, India
e-mail: chestimh2008@yahoo.co.in

J. Qadri
e-mail: qadriavid2008@gmail.com

T. N. Qadri · A. Hamid
Department of Botany, GDC Budgam, Srinagar, Jammu and Kashmir, India
e-mail: tabasumqadri@yahoo.co.in

A. Hamid
e-mail: hameed901@gmail.com

M. M. Azooz
Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt
e-mail: azzozm@yahoo.com

Department of Biological Sciences, Faculty of Science, King Faisal University,
Al Ahsa, Saudi Arabia

P. Ahmad
Department of Botany, GDC Anantnag, Srinagar, Jammu and Kashmir, India
e-mail: parvaizbot@yahoo.com

Introduction

Bio-fertilizers are microbial inoculants of bacteria, algae, fungi that augment the availability of nutrients to the plants. Use of bio-fertilizers, in contrast to chemical fertilizers, accounts economical and ecological benefits to farmers (Brahmaprakash and Sahu 2012). Different types of microorganisms show the potential of converting essential soil nutrients which are in unavailable form to available form with the help of biological activity biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi et al. 2008). Bio-fertilizers improve plant growth, protect plants from amelioration of toxic effect in soils, root pest and disease control, improved water usage and soil fertility (Halim 2009; El-yazeid et al. 2007; Badawi et al. 2011; Mader et al. 2011; Mohammadi and Sohrabi 2012).

In addition they get engaged in symbiotic as well as associative microbial activities with higher plants Tiwari et al. 2003. These being an economical and safer source of plant nutrition for increasing the agricultural production, improve soil fertility and are called mini fertilizer factories (Vyas et al. 2008). The microorganisms form root nodules in leguminous plants by colonizing roots of legumes. Nitrogen fixation, phosphorus solubilization and phytohormone production abilities have been observed and result in enhancement of agricultural productivity, e.g. *Rhizobium* for legumes (grain, fodder) (Ali et al. 2010) plant growth promoting rhizobacteria (PGPR) for cereals (wheat, rice, grasses etc.), *Azolla* for rice ecosystem, and actinomycetes (*Frankia* spp.) (Zhang et al. 2012), for forest trees (Danso et al. 1992). These microorganisms also have the ability to convert atmospheric nitrogen to plant usable form and can provide up to 200 kg N/ha/crop. Besides nitrogen, phosphorus is an essential element for crop production. Another group of bacteria which play important role in stimulating growth of plants are plant growth-promoting rhizobacteria (PGPR), they in addition to stimulating growth of plants also control plant pathogens, and pest infestation i.e they act as bio-fertilizers as well as biopesticides and ought to have meticulous consideration for agricultural purposes (Lugtenberg and Kamilova 2009). PGPR colonizes the rhizosphere, i.e, around the root, and even in the intercellular spaces of root.

Advantages of Bio-fertilizers over Chemical Fertilizers

Uses of microbial products have various advantages over traditional chemicals for agricultural purposes (Mahdi et al. 2010). These products have been commended safer than many of the chemicals that are used, they can fix atmospheric nitrogen in nodules of leguminous plants and soil and make it available to the plants and increase the fertility of soil (Shankar et al. 2012). They solubilize the insoluble forms of phosphorous and again make it available to the plants (Hashemabadi et al. 2012), they also produce hormones which promote the growth of rhizosphere in addition to these properties they also help in mineralization of soil by decomposing the organic matter (Mahdi et al. 2010).

Above all neither toxic exudates of these microbes, nor microbes themselves are accumulated in the food chain, self-replication of microbes curtails the need for repeated application and target organisms rarely build up resistance as is observed when chemical agents are used to get rid of the pests detrimental to plant development (Mahdi et al. 2010).

Agricultural land deprived of essential nutrients gets impoverished after long term cultivation, to provide or nourish the soil nutrient content under conventional farming system, farmers use apply elevated doses of chemical fertilizers which in turn contaminate the ecosystem. Thus to implement the agricultural land a balanced and accountable use of organic agriculture is required. The principles of organic farming also outline the similar concepts where the soil health and biodiversity is built up to sustain the plant growth in longer term (Mahdi et al. 2010). Various beneficial microbes and their products found in rhizosphere are useful to plants by means of promoting growth or by acting as bio-control agents or both and are termed as Plant Growth-Promoting Rhizobacteria (PGPR) (Akhtar et al. 2012; Faramarzi et al. 2012). Rosenblueth and Martinez (2006) described several endophytic bacteria from different plant species mainly belonging to genera *Rhizobium*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Azotobacter*, *Burkholderia*, *Herbaspirillum*, etc. play beneficial roles e.g. endophytic N-fixation, increased P-uptake, improve photosynthesis and plant vigor, tolerance to biotic as well as abiotic stresses and in addition to these properties they act as insecticides and help in phytoremediation of polluted soils. Bio-fertilizers application can be used on crops prior to planting i.e. directly to soil, as a side dressing or as a foliar spray because it does not pollute and it adds humus to the soil (Raj 2007; Venkataswarlu 2008). Co-inoculation of some *Pseudomonas* and *Bacillus* strains along with effective *Rhizobium* spp. is shown to stimulate chickpea growth, nodulation and nitrogen fixation (Mohammadi et al. 2010). Findings of Mohammadi et al. (2010) showed that the highest sugar, protein, starch contents, nodule weight and seed nitrogen, potassium, phosphorus of chickpea were obtained from combined application of phosphate solubilizing bacteria, *Rhizobium* and *Trichoderma* fungus. The Bio-fertilizers fix nitrogen in the soil that benefits the plant to overcome the nutritional stress. Appropriate doses of phosphorus, potassium, zinc, iron, molybdenum and cobalt along with fertilizers mitigate the stress and the legume starts responding directly to the nutrient. Usually most of the nitrogen fixed passes directly into the plant whereas some of it gets leaked into the soil for non-legume plant. However, after death, decay of these legumes by micro-organisms nitrogen eventually returns to the soil.

Types of Bio-fertilizers

A variety of recognized microorganisms for nitrogen fixation are also used such as *Azorhizobium caulinodans* and is effectively utilized in rice and maize. Likewise *Acetobacter* and *Sinorhizobium* have been used for sugarcane and soybean crop. Respectively microbes like *Thiobacillus* and *Thiooxidans* are known for sulphur and iron oxidization.

Nitrogen Fixing Bio-fertilizers

The nitrogen fixing bacteria are of two types' i.e., biological nitrogen fixation (symbiotic) and non-symbiotic nitrogen fixation (free living). The former develops as an association with crop plants through formation of nodules in their roots while as free living bacteria can fix atmospheric nitrogen without association with plants.

Biological Nitrogen Fixation

Atmosphere contains approximately 70% of N which is not readily available form and therefore is not consumed by living organisms. It can be made available with the help of chemical or biological processes, though chemical nitrogen fertilizers are relatively expensive (Zilli et al. 2004). Living organisms utilize nitrogen in the form of ammonia to synthesize proteins, nucleic acids, amino acids, and other nitrogen-containing compounds for the maintenance of life. The process of conversion of inert N₂ to biologically important NH₃ with the help of bacteria is called biological Nitrogen Fixation. The nitrogen fixation is done by the bacteria, and the NH₃ produced is absorbed by the plant.

This biological reduction of nitrogen to ammonia is performed only by some prokaryotes and is a highly oxygen-sensitive process. The Biological nitrogen fixation includes diverse range of diazotrophic soil microbes belonging to aerobes (*Azotobacter*, *Beijerinckia*, *Drexia*), facultative anaerobes (*Clostridium*, *Pseudomonas*, *Rhizobium*), heterotrophs (*Klebsiella*, *Enterobacter*), phototrophs (*Anabaena*, *Nostoc*, *Azospirillum*) The most competent nitrogen fixers establish a symbiosis with higher plants in which the energy for nitrogen fixation and, in general, the oxygen protection system in particular are provided by the plant partner. In these symbiotic relationships prokaryotic partnership is provided by soil bacteria *Rhizobium* in leguminous plants and *Frankia* bacteria in actinorhizal symbiosis. Biological Nitrogen Fixation confers tremendous amount of NH₃ to natural ecosystems.

Rhizobium

Rhizobium is a gram-negative, free living organism present in soil, once it comes in contact with specific legume crop, nitrogen fixation starts and this rhizobium-legume association is of significant environmental and agricultural importance in view of the fact that it accounts for an estimated 180 million tons biological nitrogen fixation per year (Postgate 1982). *Rhizobium* invades the root hairs of the legumes by forming nodules. First time, bacterium capable of fixing nitrogen was isolated from nodules of a legume in 1888 by Beijerinck from Holland. Later on this bacterium was reported in Bergey's Manual of Determinative Bacteriology under the genus *Rhizobium*. *Rhizobium* has the ability to fix the atmospheric N in symbiotic state only. *Rhizobium* also exists as an endosymbiotic N fixing microorganism

associated with root of legumes. It enters into plants through the root system then it forms nodule. The name Rhizobium was established by Frank in 1889. Seven distinct species of rhizobium has so far being discovered on the basis of “Cross Inoculation Group Concept” and more than twenty cross-inoculations groups have been established so far. Out of this, merely seven are most important. One group of rhizobia are very slow growing and are known as Bradyrhizobium while as other group is rhizobia is fast growing and is known as Rhizobium. Both slow growing as well as fast growing rhizobia has ability to fix atmospheric nitrogen. They create a symbiotic association with legumes and some non-legumes like Parasponia. Rhizobium legume symbiosis is very host-specific process and it fixes N in particular host plant only, this host specificity is mediated by plant compounds such as flavonoids (Goethals et al. 1992). Flavonoid activates the nod genes present in Rhizobium. The communication of rhizobia and legumes begins with signal exchange and recognition of the symbiotic partners, which is followed by attachment of the rhizobia to the plant root hairs. After infection, the root hair starts deforming, and the bacteria invades the plant by a newly produced infection thread growing through it at the same time, cortical cells which are mitotically activated, give rise to the nodule primordium. Infection threads grow toward the primordium, and the bacteria are then released into the cytoplasm of the host cells, surrounded by a plant derived peribacteroid membrane (PBM) (Van Workum et al. 1998). In the course of process the nodule primordium develops into a mature nodule, while the bacteria differentiate into their endosymbiotic form that is called as the bacteroid. The effective nodules are filled with pink sap called leghaemoglobin pigment. Leghaemoglobin regulates the supply of oxygen to the bacteria and helps the activity of nitrogenase enzyme and other regulatory enzymes (Choudhury and Kennedy 2004). The nitrogenase is responsible for reduction of nitrogen to ammonia in the process of nitrogen fixation. Bacteroids, together with the surrounding PBMs, are called symbiosomes. When symbiosomes are developed, bacteria synthesize nitrogenase, which catalyzes the reduction of nitrogen (Mylona et al. 1995). The product of nitrogen fixation, ammonia, is then exported to the plant. The plant provides all immediate nutrients and energy for the bacteria and just in a week small bead like structures i.e., nodules are formed. The root nodules act as a micro fermentor for biological N fixation where they can convert atmospheric N into ammonia. Rhizobium is able to induce the shoot and root growth in rice plants. (Yanni and El-Fattah 1999). Nodules occur in many shapes such as in *Alfalfa* and clover, nodules are fingerlike, round in *Lentil*, palm shaped in *Cicer*, though the entire nodule is generally less than 1/2 in in diameter during favorable conditions. Since the Nitrogen fixed is not free so the plant must contribute a considerable amount of energy in the form of photosynthates and other essential nutritional factors which are important for the bacteria. Rhizobium plays a key role and is the maximum researched bio-fertilizer (Mishra and Dadhich 2010). Currently the legume-rhizobia symbiosis has been extended to economically essential food crops or cereals and certain rhizobia that are competent of crack entry into ruptured epidermis during emergence of lateral rootlets in cereal crops (Kalia and Gupta 2002) the process can be improved by the addition of phytohormones (Kannaiyan et al. 2001) or use of signal chemicals (Amutha and Kannaiyan 2000).

Classification of *Rhizobium* Bio-fertilizers

1. *Rhizobium leguminosarum*
2. *Biovarphaseoli Phaseolus* (Bean)
3. *Biovarviceae Vicea* (Vetch)
4. *Biovartrifolii Trifolium* (Berseem)
5. *Rhizobium meliloti Melilotus* (Senji) *Rhizobium loti*
6. *Bradyrhizobium japonicum Glycine* (Soybean)
7. *Bradyrhizobium species Lupinus* (Lupin), *Vigna*, *Cicer*

Recently two more genera have been included in the family Rhizobiaceae. They are ***Sinorhizobium*** and ***Azorhizobium*** which are nodulating the Soybean and Dhaincha (***Sesbania***), respectively. ***Azorhizobium caulinodans*** were isolated from the stem nodules of *Sesbania rostrata* but can also colonise and produce nodules in rice roots. *Azorhizobium caulinodans* also capable of fixing nitrogen in the free living state (Mandon et al. 1998).

Blue Green Algae (BGA)/Cyanobacteria

Blue-green algae or cyanobacteria are photosynthetic prokaryotes capable of fixing nitrogen with the help of enzyme nitrogenase. They are generally aquatic, small organisms visible as a single cell or large accumulation of cells (colonies) or strings of cells i.e. trichomes under microscope, sometimes accumulations are so large that they can be seen with a naked eye. Another name for blue-green algae is cyanophytes, cyanobacteria and most recently cyanoprokaryotes. As far as vegetative structure is considered they are resemble algae and other free living bodies. Their requirements for light, nutrients and carbon dioxide are similar. Certain types of blue-green algae have tiny gas vesicles in their cells that help to regulate buoyancy or get submerged under water in response to light fluctuations and availability of nutrient. BGA include *Anabaena*, *Nostoc*, *Plectonema*, *Syctonema*, *Calothrix*, *Aulosira*, *Tolythrix*. Among these commercially available representatives are cultures of *Anabaena*, *Nostoc*, *Tolyphorix* and *Aulosira*. The blue-green alga (*Anabaena azollae*) shows a symbiotic association with *Azolla* (aquatic fern) and also fixes atmospheric nitrogen. BGA has shown to be associated with the *Azolla* present in ventral pore along the dorsal lobe of each vegetative leaf. This endophyte fixes atmospheric nitrogen and remains inside the tissue of the water fern in addition to its use in utilization in paddy fields. BGA fixes atmospheric nitrogen in semi aquatic ecosystem and takes part in photosynthetic activity. *Azolla* is a fast growing water fern and has ability to double its weight within a week. *Azolla* being rich in organic manure mineralizes the soil nitrogen rapidly and is made available to the plants. It is a protein rich feed to fish and poultry. BGA besides nitrogen fixation also synthesizes and releases growth stimulating substances viz., auxin and amino compounds that enhance the growth of rice plants. Algae can be multiplied in the paddy field by broadcasting the inoculants at the rate of about 10 kg/ha. It has been observed

that incorporation of *Azolla-Anabaena* to paddy field increases rice yields and addition of dried *Azolla filiculoides* at the rate of 93 kg N/ha has increased a rice yield upto 70%, the increase obtained with an equivalent amount of ammonium sulphate (Anitha and Kannaiyan 1999). In a field experiment the cyanobacteria was used to degrade coir pith with the help of lignolytic enzyme. (Malliga et al. 1996) and produced cyanopith, it can be used as bio-fertilizer to improve the crop productivity (Jha and Prasad 2005). Coir pith contains high lignin (31%), cellulose (27%), content (Bhat et al. 2003) and carbon nitrogen ratio (C/N) of 104:1 (Palaniappan 2005). Manoharan et al. 2011 used cynopith as bio-fertilizers on *Amaranthus dubius* that increases the growth of *Amaranthus*.

Azospirillum

Azospirillum is microaerophilic, free living, non-symbiotic, loosely associative nitrogen fixing bacteria and it establishes a close association with various plants mainly with C₄ maize, sorghum, sugarcane, ray grass, *Amaranthus* etc. This micro-organism fixes atmospheric N and makes it available for plants in asymbiotic manner (Steenhoudt and Vanderleyden 2006). *Azospirillum* grows in the rhizosphere of the plants or occasionally penetrates into the root tissues but is not able to produce any visible nodule or out growth on the root tissue but grows intracellularly (Saikia et al. 2007). This association is due to the ability of the microbe to use malic acid, an organic acid formed for capturing CO₂ as a carbon source. It also secretes various phytohormones which include gibberellins, cytokinins, auxins and affect development and morphology of root by increasing root length, number of root hair cells, lateral roots. *Azospirillum* also secretes iron-chelating siderophores that help in the sequestering of iron sufficient for plant growth (Romerheld and Marshner 1986). A free living nitrogen fixing bacteria was for the first time reported by Beijerinck in 1925 under the name of *Spirillum lipoferum* and later on renamed this organism as *Azospirillum* (nitrogen fixing *Spirillum*) in 1978. *Azospirillum* is one of recognized dominant soil microbe and is able to fix about 10–40 kgN/ha. The *Azospirillum* inoculation improves vegetative growth of the plants (Naderifar and Daneshian 2012). Till date only four species of *Azospirillum* have been identified which include *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. iraquense*. Among these species only *A. brasilense* and *A. lipoferum* are very common in Indian soils. Inoculation of vegetable crops with *Azospirillum* has resulted in yield enhancement. The field experiment of *Azospirillum* with maize was examined and was confirmed that this association benefits enzyme activating glutamine synthetase and glutamine synthetase in the leaves of paranodulated maize plants. Bhaskara Rao and Charyulu (2005) studied the association of *A. lipoferum* inoculated to foxtail millet plant in combination with N fertilizer and demonstrated the increase in plant growth level, dry weight of shoot and root over when compared with control plants.

Maize plants inoculated with *Azospirillum* showed high rate of photosynthesis and stomatal conduction leading to high yield compared to control plants (Kumar

and Bhaskara Rao 2012). Rice plant inoculated with *A. brasilense* at population rate of 8×10^{-7} /g of dry weight under field conditions showed a yield of 1.6–10.5 g plant⁻¹ (Mirza et al. 2000; Malik et al. 2002). *Azospirillum* population enhances the uptake of P and NH₄ compounds in rice plants (Murty and Ladha 1988).

Azotobactor

Azotobactor is a gram-negative, aerobic, heterotrophic, rod shaped nitrogen fixing bacteria present in alkaline and neutral soils (Lakshmi-narayana 1993). They are free living organism present in soil, water and also in association with some plants (Gandora et al. 1998; Martyniuk and Martyniuk 2003). Various species of *Azotobacter* are *A. agilis*, *A. chroococcum*, *A. beijerinckii*, *A. vinelandii*, *A. ingrinis*. Among these *Azotobacter*, *Azotobacter chroococcum* is the most commonly found in arable soils of India. In addition to its capability to fix atmospheric nitrogen (20–40 Kg N/ha) for different crops, it can also produce various growth promoting substances viz., auxins, and gibberellins cytokinins, indole acetic acid including vitamins and antibiotics, which control plant pathogens and help to maintain soil fertility. *Azotobacter* produces slime like substances which help in aggregation of soil particles. Many strains of *Azotobacter* exhibit fungicidal properties against certain species of fungus. Various crop plants like rice, maize, cotton, sugarcane, pearl millet, vegetable and some plantation crops show response to *Azotobacter*. Occurrence of organic matter in uncultivated soil promotes its multiplication and nitrogen fixing capacity. Field experiments carried out on *Azotobacter* under different agro-climatic conditions pointed out that *Azotobacter* is suitable when inoculated with seed or seedling of crop plants like onion, brinjal, tomato and cabbage. *Azotobacter* being heaviest among breathing organism and requires a large amount of organic carbon for its growth. Although it is poor competitor for nutrients in soil but it enhances plant growth through nitrogen fixation, release of growth promoting substances, and fungicidal substances. It improves seed germination and plant growth. N fixation process which is highly sensitive to O₂, *Azotobacter* have special mechanism against O₂ it reduces the concentration of O₂ in the cells (Shank Yu et al. 2005). Nitrogenase enzyme is also sensitive to O₂, but is supposed that the extreme respiration role of *Azotobacter* utilizes free O₂ within the cells and protects the nitrogenase (Kumar and Bhaskara Rao 2012). *Azotobacter* species have various types of nitrogenases viz., molybdenum–iron nitrogenase, vanadium–iron nitrogenase (Robson et al. 1986; Narula et al. 2000). *Azotobacter* requires carbon source for their energy (Kanungo et al. 1997) and is capable of fixing 10 mg N/g of carbohydrates in field conditions. *Azotobacter* is believed to be one of the significant bio-fertilizer for rice and other cereals, it can be applied by seed dipping and seedling root dipping methods (Kannaiyan et al. 1980; Kannaiyan 1999; Ruttimann et al. 2003; Singh et al. 1999). *Azotobacter* can also able to enhance the growth in wheat crop (Kader et al. 2002).

Non-legume-Frankia Symbiosis

Frankia a genus of actinomycetes, is a free, gram's positive nitrogen fixing bacterium that lives in soil and develops symbiotic interaction with various trees and shrubs forming symbiotic nodules (Vergheze and Misra 2002). There are about 264 species belonging to 25 genera which take part in Frankia symbiosis. The Frankia is of fundamental and ecological interests for diverse reasons that include its wide distribution, its ability to fix nitrogen, differentiate specialized cell for nitrogen fixation (Vergheze 2002). These specialized cells are called sporangium and vesicles and in addition to it can nodulate non-leguminous trees by forming root nodules, such as *Casuarina*, *Alnus*, *Dansea*, *Myrica*, *Elaeagnus* (Dawson et al. 2005; Franche et al. 2009). In wastelands fertility of soil can be improved by growing such non-leguminous plants in nitrogen deficient soils. In the process of nodulation, *Frankia* develops as little lateral swelling on roots and subsequently develops into new lobes at their apices forming cluster coralloid structure (Duhoux et al. 2001). Inoculation of *Frankia* enhances growth, nodulation, nitrogenase activity of nodule and nodule dry weight of *Casuarina* and *Alnus* plants. They live in the soil and have a symbiotic relationship with certain woody angiosperms, called actinorhizal plants. *Frankia sp.* produces three types of cells: sporangiospores, hyphae, and diazo-vesicles (Tjepkema et al. 1980), these diazo-vesicles are spherical, thick walled, lipid-enveloped cellular structures responsible for providing sufficient nitrogen to the host plant during symbiosis. *Frankia* enter into plants by root hair infection, nodules formed on lateral roots with cortical cylinder of vascular tissue (Ganesh et al. 1994). *Frankia* supplies almost total nitrogen needed by host plant and thus can establish a nitrogen-fixing symbiosis with host plants where nitrogen is the limiting factor for plant development. Therefore, actinorhizal plants colonize and often prosper in soils that are low in combined nitrogen (Benson and Silvester 1993). Symbiotic interaction of this category adds a large quantity of new nitrogen to numerous ecosystems such as temperate forests, dry chaparral, sand dunes, mine wastes etc. They also assist in creating and transporting certain root hormones, controlling pathogens and nematodes, water retention, mineral uptake, root exploration and resource sharing (Benson and Silvester 1993). *Frankia* specifically fixes nitrogen in the air and produces molecules that other plants can use. *Frankia* is said to be responsible for 15% of the biologically fixed nitrogen in the world (Trujillo 2008).

Plant Growth Promoting Rhizobacteria

An assemblage of rhizobacteria (bacteria on rhizosphere) to facilitate beneficial effect on plant growth is referred to as plant growth promoting rhizobacteria or PGPR (Schroth and Hacock 1981). PGPR belong to several genera, e.g., *Alcaligenes*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Actinoplanes*, *Bacillus*, *Bradyrhizobium*, *Amorhosporangium*, *Pseudomonas sp.*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Cellulomonas*, *Streptomyces*, *Flavobacterium*, and *Xanthomonas* (Weller 1988). In a recent study it was found that PGPR covers a wide range of plant species. In all

successful plant microbe interactions, the capability to colonize plant habitats is important. Single bacterial cells can affix to surfaces and, after repeated cell divisions and proliferation, dense aggregates are formed which are commonly referred to as macro colonies or biofilms (Mohammadi and Sohrabi 2012). Steps of colonization include attraction, recognition, adherence, invasion (only endophytes and pathogens), colonization and growth, and several strategies to establish interactions (Nihorimbere et al. 2011). There is crosstalk between plant roots and soil microbes. Plants roots initiate crosstalk by producing signals that are recognized by the microbes, which in turn produce signals that initiate colonization (Berg 2009). PGPR reach root surfaces by active motility facilitated by flagella and are guided by chemotactic responses. This implies that PGPR capability highly depends either on their abilities to take advantage of a specific environment or on their abilities to adapt to changing conditions or plant species (Nihorimbere et al. 2011). Habibi et al. (2011) strongly recommended that use of bio-fertilizers (combined strains) in addition with organic and chemical fertilizers have resulted in the maximum grain yield and oil yield in medicinal pumpkin. They revealed that 50% of required nitrogen and phosphorus fertilizers might be replaced by bio and organic fertilizers, since bio and organic fertilizers improve the efficiency of recommended nitrogen and phosphorus fertilizers and reduced the cost of chemical fertilizers and also prevent the environment pollution from extensive application of chemical fertilizers. de Freitas et al. (1993) demonstrated that inoculation of beans with *Rhizobium leguminosarum* and *Pseudomonas putida* increased the number of nodules and acetylene reduction activity (ARA) significantly. A significant positive effect on grain yield and ARA in roots of barley was obtained due to combined inoculation of nitrogen fixer's *A. lipoferum*, *Arthrobacter mysorens* and the phosphate solubilizing strain *Agrobacterium radiobacter* by Belimov et al. (1995). Radhakrishnan (1996) revealed that inoculation of *Azospirillum* and phosphor-bacteria resulted in higher root biomass and more bolls in cotton. Findings of Mohammadi (2010) showed that inoculation of bio-fertilizers (PSB + *Trichoderma* fungi) + application of FYM had a great influence on canola growth, height and grain yield when compared to control treatment. Findings of Mohammadi et al. (2011) showed that application of bio-fertilizers had a significant effects on nutrient uptake of chickpea combined application of Phosphate solubilizing bacteria and *Trichoderma harzianum* produced the highest leaf P content and grain P content. Capacity of *Bacillus* sp. to produce organic acid such as gluconic, citric and fumaric acids under P-limiting conditions may increase the solubility of poorly soluble phosphorus (Mohammadi and Sohrabi 2012).

Phosphorus Solubilizing/Mobilizing Microorganisms (PSM)

Phosphorous makes about 0.2% of the plant on dry weight basis. It has distinct role in plant metabolism which includes cell division, cell development, photosynthesis, breakdown of sugars, nuclear transport within plants, and transfer of genetic characteristics from one generation to another generation and regulation of metabolic

pathway (Rodriguez and Fraga 1999). The plant obtains their phosphate requirements from the soil pool. It occurs in soil as inorganic phosphate, produced by weathering of rocks that is unable to be utilized by the plants (Lee et al. 2005) or as organic phosphate derived from decaying plant, animal or microorganisms (Rodriguez and Fraga 1999). About 15–20% of applied phosphorus is recovered from the crops and rest gets fixed in the soil and is not readily available to the plants. A group of morphologically different microorganisms which have the property of solubilizing the fixed phosphorous by producing organic acids and enzymes and to make them easily available to the crops are known as Phosphorous Solubilizing Microorganisms (PSM). They include diverse species of *Bacillus*, *Aspergillus*, *Pseudomonas*, *Penicillium*, *Agrobacterium*, *Achromobacter*, *Burkholderia*, *Aerobacter*, *Erwinia*, *Micrococcus*, *Flavobacterium* and *Trichoderma*. These organisms solubilize the fixed soil phosphorus thereby releasing the citrate and water soluble phosphorus so as to help in mineralizing organic phosphate compounds that are present in the organic wastes (Rodriguez and Fraga 1999). These microorganisms have the property to bring phosphate solubilization by secreting organic acids such as propionic acid, lactic acid, formic acid, acetic acid, succinic acids etc. these acids lower the pH and help to dissolve the phosphate bound (Rodriguez and Fraga 1999). They also produce growth promoting substances e.g. IAA, GA etc. experiments conducted in field conditions in India have shown to replace 20–50 kg P_2O_5 /ha in different crops due to PSM's inoculation (Vora and Shelat 1996, 1998, 1999). Improvement in seed germination by application of PSB has been reported by Sharma et al. (2007) in *Cicer arietinum*. Various horticultural plants and vegetables were successfully inoculated with P-solubilizing bio-fertilizers to obtain higher yields (Khan et al. 2010; Velineni and Brahmaprakash 2011). Field experiments demonstrated that P-solubilizing bio-fertilizers in addition to improving the growth and quality of crops, also reduced the usage of chemical or organic fertilizers significantly (Young 1990; Chang and Young 1992a, b; Young et al. 1998a, b; Young and Chen 1999; Chang and Young 1999; Young et al. 2000; Liu and Young 2001; Young et al. 2003). Phosphate solubilizing bacteria has the capacity to convert inorganic unavailable phosphorus form to soluble forms like HPO_4^{2-} and $H_2PO_4^-$ with the help of processes like organic acid production, chelation and ion exchange reactions and make them available to plants (Chang and Yang 2009; Banerjee et al. 2010). Naturally occurring rhizospheric phosphorus solubilizing microorganism (PSM) has a long history and dates back to 1903 (Khan et al. 2007). Alam et al. (2002) pointed out that bacteria are more effective in phosphorus solubilization than fungi. Among the whole microbial population in soil, phosphate solubilizing bacteria (PSB) comprise 1–50%, whereas phosphorus solubilizing fungi (PSF) are only 0.1–0.5%. (Chen et al. 2006). Number of phosphorous solubilizing bacteria amongst total PSM in north Iranian soil was around 88% (Fallah 2006). Microorganisms concerned in phosphorus acquirement include mycorrhizal fungi and PSMs (Fankem et al. 2006). Among the soil bacterial communities, effective phosphate solubilizers ectorrhizospheric strains from *Pseudomonas* and *Bacilli*, and endosymbiotic rhizobia have been described as (Iguar et al. 2001). Strains from bacterial genera *Pseudomonas*, *Bacillus*, *Rhizobium* and *Enterobacter* along with *Penicillium* and *Aspergillus* fungi

are the most influential P solubilizers (Whitelaw 2000). *B. circulans*, *Bacillus megaterium*, *B. subtilis*, *B. sircalmous*, *B. polymyxa*, *Enterobacter* and *Pseudomonas striata*, can be referred as the most important strains (Subbarao 1988; Kucey et al. 1989). A fungus *Arthrobotrys oligospora* is also found to have the ability to solubilize the phosphate rocks (Duponnois et al. 2006). Increased high percentage of PSM is concentrated in the rhizosphere, and they are metabolically more active than from other sources (Vazquez et al. 2000). By and large, 1 g of fertile soil contains about 101–1010 bacteria, and their live weight may exceed 2,000 kg ha⁻¹. Soil bacteria can be cocci (sphere, 0.5 μm), bacilli (rod, 0.5–0.3 μm) or spiral (1–100 μm) shapes. Bacilli are common in soil, where as spirilli are very rare in natural environments (Baudoin et al. 2002). The PSB are cosmopolitan and vary in forms and population in diverse soils. Their population depends upon the physical and chemical properties organic content and phosphorous content of soil and cultural activities (Kim et al. 1998). Maximum populations of PSB are found in agricultural and rangeland soils (Yahya and Azawi 1998). In north of Iran, the PSB count ranged from 0 to 107 cells g⁻¹ soil, with 3.98% population of PSB among total bacteria (Fallah 2006). Mineralization and solubilization potential for organic and inorganic phosphorus, are also shown by bacterial populations (Hilda and Fraga 1999; Khiari and Parent 2005). Phosphorus solubilizing activity is determined by the capacity of microbes to liberate metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelate the cation bound to phosphate, than are transformed to soluble forms (Sagoe et al. 1998). Various microbial processes/mechanisms including organic acid production and proton extrusion are used in Phosphate solubilization. (Surange 1995; Dutton and Evans 1996; Nahas 1996). A wide range of microbial P solubilization mechanisms exist in nature and much of the global cycling of insoluble organic and inorganic soil phosphates is attributed to bacteria and fungi (Banik and Dey 1982). Whitelaw (2000) suggested that Phosphorus solubilization is also carried out by a large number of saprophytic bacteria and fungi acting on sparingly soluble soil phosphates, mainly by chelation-mediated mechanisms. Phosphate solubilizing microorganisms secrete organic acids and enzymes that act on insoluble phosphates and convert it into soluble form, thus, proving P to plants (Ponmurugan and Gopi 2006). Inorganic P is solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelate cations (Al, Fe, Ca) and decrease the pH in basic soils (Kpombekou and Tabatabai 1994; Stevenson 2005). The PSB dissolve the soil P through production of low molecular weight organic acids mainly gluconic and ketogluconic acids (Goldstein 1995; Deubel et al. 2000), in addition to lowering the pH of rhizosphere. The pH of rhizosphere is lowered through biotical production of proton/bicarbonate release (anion/cation balance) and gaseous (O₂/CO₂) exchanges. Phosphorus solubilization ability of PSB has direct correlation with pH of the medium. In addition to phosphorous solubilization ability of PSB, they also can improve plant growth by enhancing the availability of other trace element such as iron (Fe), zinc (Zn), etc. Gull et al. (2004) suggested that PSB can solubilize the fixed soil P and applied phosphates resulting in higher crop yields. According to Goenadi et al. (2000) direct application of phosphate rock is usually ineffective in the short time period of most

annual crops. Gyaneshwar et al. (2002) suggested that acid producing microorganisms are able to increase the solubilization of phosphatic rock. The PSB in conjunction with single super phosphate and rock phosphate reduce the P dose by 25 and 50%, respectively (Sundara et al. 2002). *Pseudomonas striata* and *Bacillus polymyxa* solubilized 156 and 116 mg P L⁻¹, respectively (Rodríguez and Fraga 1999). *Pseudomonas fluorescens* solubilized 100 mg P L⁻¹ containing Ca₃(PO₄)₂ or 92 and 51 mg P L⁻¹ containing AlPO₄ and FePO₄, respectively (Henri et al. 2008).

Mycorrhiza

Mycorrhizae are mutualistic associations between fungi and plant roots. The host plant gets mineral nutrients from mycorrhizal fungi, while as the fungus partner is provided with photosynthetic products from the host plant (Jakobsen et al. 2002). Fungi become integrated into the root structure, or fungi lives in close association with plant roots. Fungal hyphae may live on the external surface of roots (ectomycorrhizal) or may invade root cells (endomycorrhizal). Mycorrhiza belong to fungi kingdom Basidiomycetes, Ascomycetes and Zygomycetes. Mycorrhizal fungi, and fungi generally, have a strong influence on soil structure (Rillig and Mummey 2006). Their hyphal strands help to hold soil aggregates together, and they also excrete organic substances that help cement the aggregates (Rillig and Mummey 2006). Hyphae conduct water and immobile nutrients (like P) to roots despite disruption of capillary water flow in soil. Of the many types of mycorrhizal association the most important association which are economically as well as ecologically importance are: ectomycorrhizal associations, and the endomycorrhizal association of the vesicular-arbuscular (VA) type (Rillig and Mummey 2006). In case of ectomycorrhizal associations, the fungi attack the cortical region of the host root devoid of piercing cortical cells. Ectomycorrhizae are recognized to occur in the families of Salicaceae, Fagaceae, Pinaceae, Betulaceae, Tiliaceae, Juglandaceae and Ceasalpinionideae. The ectomycorrhizal roots lack root hairs and are covered by a sheath of fungal hyphae which almost looks like host tissue. This tissue is called Pseudoparenchymatous sheath. Hyphae from this sheath enter into the cortex and remain in the outer cortical region to form a network called Hartig's net (Alizadeh 2011). The nutrients absorbed by the hyphae are transported to the plant with the help of this Hartig's net. Infection of host plants by ectomycorrhizal fungi frequently leads to changes in feeder roots that are apparent to the naked eye but in case of endomycorrhizal associations of the VA type, the fungi penetrate the cortical cells and form clusters of delicately divided hyphae known as *arbuscules* in the cortex (Alizadeh 2011). They also form vesicles, which are membrane-bound organelles of varying shapes, inside or outside the cortical cells. Arbuscules are supposed to be the sites where resources are exchanged among the host plant and the fungi (Alizadeh 2011). Vesicles in general serve as storage space but when they are old they can serve as reproductive structures. Vesicles and arbuscules, together with large spores, comprise the diagnostic features of the VA mycorrhizas. Most ectomycorrhizal fungi

belong to several genera within the class Basidiomycetes, while some belong to the zygosporic Zygomycetes and Ascomycetes. On the other hand, AM fungi belong to six genera within the azygosporous zygomycetes

Vesicular Arbuscular Mycorrhiza (VAM)

VAM are common, ancient and most fascinating class of fungi which proves to be very beneficial to plants (Alizadeh 2011). VAM is an endotrophic mycorrhiza formed by aseptate phycomycetous fungi. They produce an interconnected network of hyphae between cortical cells that extend to the soil and hence absorb various nutrients and water (Sally et al. 2011) VAM forms an association with various crop plants which include monocot, dicot, annual or perennial crops. The use of VAM enhances growth of plants in less fertile soils besides application of FYM and cereal-legume crop rotations. Whereas, application of chemicals mostly fungicide suppresses its existence.

Mycorrhiza enhances the feeding areas of the plant root as the hyphae spreads around the roots. It also mobilizes the nutrients particularly phosphorous that are present in organic or inorganic form in soil and translocate it to plants with the help of extensive mycelium. In addition to translocation of phosphorous to plant it also stores the nutrients and removes the toxic substances for example, phenolics which otherwise hinder nutrient availability in addition to this it also provides protection against other fungi and nematodes. VAM also assists in transfer of nutrients other than phosphorus, like zinc and sulfur Cu (copper), K (potassium), Al (aluminum), Mn (manganese), Fe (iron) and Mg (magnesium) from the soil to the plant roots. They act as intracellular obligate fungal endo-symbiont by penetrating the root cortex (Alizadeh 2011). In addition they possess vesicles intended for storage of nutrients and arbuscular for transferring these nutrients into root system as well as enhances water absorption. However, in ecto-mycorrhiza, the hyphae cover both outside and within the root in the intercellular spaces of epidermis and cortex. Trees are usually found to be infected with ectomycorrhiza, they increase the tolerance of plants against drought and salt stress, increase the photosynthetic activity of plants, higher chlorophyll content, higher leaf water potential restored capacity (Wang 1989, 1998). VAM helps in soil conservation and soil aggregation, increase the resistance of plants against root-pathogens, increases habitatrestoration (Dodd 2000).

Conclusions and Future Prospects

Bio-fertilizers increase crop productivity by increasing availability or uptake of nutrients through solubilization or increased absorption stimulation of plant growth with the help of hormonal action or antibiosis, or by decomposition of organic residues. Moreover, bio-fertilizers also help to reduce the use of chemical fertilizers

which in turn reduces the amount and cost of chemical fertilizers and thus prevents the environment pollution from extensive application of chemical fertilizers.

To get better productivity of agricultural lands and to maintain this productivity, the integrated approach to determine the most favorable plant-microorganism interaction is important. The bio-fertilizers are thought to be more expensive and show unpredictable performance. Besides, the effect on the crops is slow, compared to chemical fertilizers. In order to get potential benefit from bio-fertilizers in commercial agriculture, consistency in their performance is to be improved. Special care such as mode of application on crops and to keep them effective for extensive use is needed. As bio-fertilizers contain living organisms, their concert therefore depends on environment surrounding them, Short shelf life, lack of suitable carrier materials, susceptibility to high temperature, problems in transportation and storage of bio-fertilizers are major bottlenecks that are at a standstill and have to be solved in order to acquire efficient inoculation. The main criteria to take into consideration in making of bio-fertilizers are microbes' growth profile, types and optimum condition of organism, and formulation of inoculums, methods of application and storage of the product are all critical to the success for a sustainable agriculture.

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