Chapter 1
Introduction
Agricultural practices that improve soil quality and agricultural sustainability have received much attention from researchers and farmers. One current proposition towards solving agro-environmental problems is integrated nutrient management (INM). It appears to be the sole alternative in bridging the gap between nutrient requirement and supply in intensive cropping systems. The INM system promotes low chemical input but improved nutrient-use efficiency by combining natural and manmade sources of plant nutrients in an efficient and environmentally prudent manner. This will not sacrifice high crop productivity in the short term nor endanger sustainability in the long term (Gruhn et al. 2000; Adesemoye et al. 2008). Integration of different sources of nutrients has a promising efficient soil health management and sustained productivity. Integrated nutrient management enables the adaptation of the plant nutrition and soil fertility management from farming system to site characteristics, taking advantage of the combine and harmonious use of organic, mineral and biofertilizers, nutrient resources to serve the concurrent needs of food production and economic environmental and social viability (Roy et al. 2006). The integrated approach achieves the health of soil and plants environment.

Nutrient availability and its absorption by plants are regarded as the greatest driver of the plant growth. Plants grow due to the nature of the soil, available nutrients, prevailing environmental conditions, pH and the particular plant in question (Kiramani et al. 2011). Depending on its locations, soils are made up of combination of some particles which include: sand, clay, silt and organic matter. Soil texture also referred to as the makeup of the soil, as well as the pH, determines the nutrient availability to plants (Parry 2010), while the former determines how well nutrient is retained in the soil as well as water, the later tend to affect the availability of nutrients in the soil. Macronutrient seems to be less available at low pH while micronutrients appreciate such conditions and vice versa. An ideal soil should therefore able to contain an equivalent component of the nutrients for an optimal growth of the plants. However, this does not naturally occur providing for soil variation in nutrients and texture, thereby making the soils more productive than the
Introduction

others. In intensive cropping system, supplementing soil nutrients by the use of chemical fertilizer is considered inevitable for obtaining optimum yield of crops. However, their utilization efficiency remains low, due to loss by volatilization, denitrification, leaching and conversion into unavailable forms. Now it is well established that continuous use of chemical fertilizers subverts the soil ecology, disrupts environment, degrades soil fertility and shows consequent effect on human health (Ayala and Rao 2002). Therefore, large-scale application of rhizobacteria to crops as inoculants would be attractive as it would substantially reduce the use of chemical fertilizers and pesticides which often pollute the environment. In addition, the application of rhizobacteria would increase crop yield, thereby helping to feed the growing world population to ensure food security to all (Pinton et al. 2001).

1.1 Plant growth promoting rhizobacteria
Soil microorganisms constitute a large dynamic source and sink of nutrients in all ecosystems and play a major role in nutrient cycling (Cambardella and Eliott 1992; Collins et al. 1992), soil structure (Lynch and Bragg 1985), reduction in phytopathogens, and other alteration in soil properties influencing plant growth and development. Differences in microbial characteristics and their properties are restricted to general ecological enumerations which are limited in their ability to perform adequately in a stressed ecosystem. Certain groups of soil-inhabiting bacteria including plant-growth-promoting rhizobacteria (PGPR) are one of the most sensitive biological markers available that has been found quite beneficial for most disturbed and contaminated ecosystems. A multiplicity of micro-organisms and their functioning is required to create soils and maintain fertility through complex cycles and interactions.

Interaction of plant growth promoting rhizobacteria (PGPR) with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region (Dutta and Podile 2010). PGPR seemed as successful rhizobacteria in getting established in soil ecosystem due to their high adaptability in a wide variety of environments, faster growth rate and biochemical versatility to metabolize a wide range of natural and xenobiotic compounds. Cook (2002)
considered PGPR as the significant component in the management of agricultural practices with innate genetic potential. The concept of PGPR has now been confined to the bacterial strains that can fulfill at least two of the three criteria such as aggressive colonization, plant growth stimulation and biocontrol (Weller et al. 2002; Vessey 2003). The term Plant Growth-Promoting Rhizobacteria (PGPR) was coined over three decades ago, they are non-pathogenic, strongly root colonizing bacteria on the surface of plant’s roots which increase plant’s yield by one or more mechanisms (Babalola 2010). Plant growth promoting rhizobacteria can affect plant growth by different direct and indirect mechanisms (Glick 1995). PGPR influence direct growth promotion of plants by fixing atmospheric nitrogen, solubilizing insoluble phosphates, secreting hormones such as IAA, GAs, and Kinetin besides ACC deaminase production (Glick et al. 1999), which helps in regulation of ethylene, Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism, production of metabolites (hydrogen cyanide, siderophores), suppressive to deleterious rhizobacteria are some of the mechanism that indirectly benefit plant growth as shown in Fig. 1.1.

PGPR can change the plant physiology and certain nutritional and physical properties of rhizospheric soil and indirectly influence on the colonization patterns of soil microorganisms in that particular region. Inoculation of rhizobacteria increased uptake of nutrient elements like Ca, K, Fe, Cu, Mn and Zn by plants through stimulation of proton pump ATPase (Mantelin and Touraine 2004). Reports are available on the combinations of Bacillus and Microbacterium inoculants to improve the uptake of the mineral elements by crop plants (Karlidag et al. 2007). This increase in nutrient uptake by plants might be explained through organic acid production by the plants and PGPRs, decreasing the soil pH in rhizosphere. Ample evidences (Phillips 1980; Forde 2000; Glass et al. 2002) are there on the maintenance of soil fertility by the rhizobacterial isolates to increase the availability of nutrients for plants. Solubilization of unavailable forms of nutrients is one of the essential criteria in facilitating the transport of most of these nutrients (Glick 1995).
Efficient capture of nutrients from soil by roots is a critical issue for plants given that in many environments nutrients have poor availability and may be deficient for optimal growth. Whilst nutrient supply in soil is often augmented by the application of fertilizers, the availability of nutrients is governed by a wide range of physico-chemical parameters, environmental and seasonal factors and biological interactions. Competition for nutrient uptake across different plant species, between different roots and with microorganisms is also significant (Hodge 2004). Biochemical changes in the rhizosphere and interaction with microorganisms are also significant. However, the importance of different root traits and rhizosphere-mediated processes is dependent on the nutrient in question and other factors that include plant species and soil type (Tinker and Nye 2000).
1.2 Plant nutrients

These are elements that are essential for plant growth and that are taken up from the soil or from water-irrigation, flood or groundwater-or are supplied via a hydroponic medium. The primary nutrients are nitrogen, phosphorus and potassium which are consumed in relatively large amounts. Three secondary nutrients are taken up in smaller quantities but are essential for plant growth: calcium, magnesium and sulphur. Micronutrients or trace elements are required in very small quantities but are important for plant or animal metabolism. They include iron, zinc, manganese, boron, copper, molybdenum and chlorine. In addition, some plants benefit from the presence of sodium, cobalt and silicon but these do not rank as essential nutrients (Brahmaprakash and Sahu 2012).

1.2.1 Nitrogen

Nitrogen (N)’s most recognized function in the plant is its presence in the protein molecules. Besides, some important molecules such as purines, pyrimidines, and the coenzymes also contain N. Nitrogen deficiency in plants causes yellowing or chlorosis of leaves in the more mature leaves. In severe deficiency, the lowermost leaves on a plant become dry and yellow. N deficiency is found in many plants in the production of pigments other than chlorophyll when N is lacking. Its supply is met by both biological and chemical fertilizers. Biofertilization by PGPR accounts for the increased N supply by approximately 65% (Maheshwari et al. 2012).

Nitrogen in the soil can also be temporarily tied up by the microbial biomass, in a process referred to as immobilization. Soil microbes require N to decompose crop residues and can get this either from the residue or soil solution. Residues with higher carbon to nitrogen ratios and more lignin, like cereal straw, decompose more slowly, immobilizing N for longer periods. Eventually decomposition will slow and microbial biomass will release the N, increasing plant available N. Biological nitrogen fixation, that is, the assimilation of atmospheric nitrogen in form of organic compounds, is a sustainable source of nitrogen in cropping systems, as fixed nitrogen can be used directly by the
Introduction

plant, and it is less susceptible to volatilization, denitrification, and leaching (Jensen and Hauggaard-Nielsen 2003; Garg and Geetanjali 2007). Thus, biological nitrogen fixation in agrosystems can mitigate the use of fertilizers and consequently reduce global warming and water contamination (Bohlool et al. 1992). Rhizosphere associated N-fixing bacteria have increasingly been used in non-legume crop species such as sugar beet, sugar cane, rice, jatropha, maize, and wheat (Sahin et al. 2004). Due to the high energy requirement for N fixation and relatively low metabolic activity of free living organisms that must compete for root exudates outside a nodule environment, the ability of nonsymbiotic bacteria to fix significant quantities of N is limited. The presence of a diazotrophic bacterium in the rhizosphere of a certain plant is no longer considered to imply that such bacteria make a substantial contribution to N fixation and N supply for plant growth. Although the N fixing capacity of certain bacteria can easily be demonstrated under in vitro conditions, its demonstration in greenhouse and field studies is more complex and highly variable. Some observations suggest that rhizobacteria can provide crops with significant quantities of N (Dobbelaere et al. 2003).

1.2.2 Phosphorus

Phosphorous (P) plays a key role in plant growth and is the major plant growth-limiting nutrient despite its abundance in soils in both inorganic and organic forms (Gyaneshwar et al. 1999). It is absorbed by the plants, in the orthophosphate (\(\text{H}_{2}\text{PO}_{4}^{-}\) and \(\text{HPO}_{4}^{2-}\)) forms (Hinsinger 2001). Phosphorus is a structural component of many co-enzymes, phospho-proteins, phospholipids (Ozanne 1980) and part of the genetic memory “DNA” of all living things. It is involved in the transfer and storage of energy which is used for growth and reproduction (Griffith 1999). Phosphorus is important in several physiological processes of plants, especially in photosynthesis, carbon metabolism, and membrane formation (Wu et al. 2005). Phosphorus plays vital role in root elongation, proliferation and its deficiency affects root architecture (Borch et al. 1999; Williamson et al. 2001), seed development and normal crop maturity. Phosphorus is readily translocated within the plants, moving from older to younger tissues as the plant forms cells and develops roots, stems and leaves. Although the total amount of P in the soil may be high, it is mainly (>80%) present in forms unavailable to plants because of adsorption,
precipitation, or conversion to organic forms. In acidic soils, P forms iron/aluminium (Fe/Al) phosphates and gets adsorbed to Fe/Al oxides or humic substances. In alkaline calcareous soils, P is often precipitated as calcium (Ca)-P. Plants growing in P-deficient soil allocate a greater proportion of assimilates to root growth and tend to have fine roots of a small diameter and therefore, a large surface area. Fine roots and especially root hairs (Gahoonia et al. 2001; Nigussie et al. 2003), are effective in scavenging P from the soil environment because of a large surface area of contact with the soil.

Microbial-mediated solubilization and mineralization of inorganic and organic P are important processes whereby microorganisms are able to acquire P from soil, it has been argued that they are unlikely to mobilize sufficient P above their own requirements to meet plant demand (Tinker 1980). Indeed, few studies have unequivocally demonstrated a direct release of P by microorganisms in soil and benefits to plant nutrition are therefore often inferred. Nevertheless, the cycling of P within the microbial biomass and its subsequent release is paramount to the P cycle in soil and represents an important pathway for movement of P from various soil pools into plant-available forms and may also serve to protect orthophosphate from becoming unavailable in soil due to various physicochemical reactions (Magid et al. 1996; Oberson et al. 2001). The significance of this in the rhizosphere permits further research. Phosphate-solubilizing bacteria use different mechanism(s) to bring about the insoluble forms of the phosphate into soluble forms. Organic acids released by the microorganisms act as good chelators of divalent cations of Ca\(^{2+}\) accompanying release of phosphates from insoluble phosphatic compounds. Organic acids may also form soluble complexes with metal ions associated with insoluble ‘P’, thus releasing the phosphate. Many of the PSMs lower the pH of the medium either by H\(^+\) extrusion (Illmer and Schinner 1995) or by secretion of organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic and citric acids (Fig. 1.2).

In soils a large amount of P is found in organic forms (Dalai 1977). This organic P (Po) is varied, complex and uncharacterized (Anderson 1980). The largest characterized fraction of Po consists on derivatives of inositol hexaphosphate (IHP) (Dalal 1977). The
phosphatases released by microorganisms or plants hydrolyze Po to inorganic forms (Cosgrove, 1967; Beck et al. 1989). Phosphatase activity in soil initiates from different sources including plant roots (Dinkelaker and Marschner 1992), fungi (Tarafdar et al. 1988) mycorrhizal fungi (Bowen 1973; Tarafdar and Marschner 1994) and bacteria (Tarafdar and Claassen 1988). Phosphatase enzymes would work effectively for hydrolyzing Po in the presence of substrate. Mechanisms which allow solubility of Po to be increased include increases in rhizosphere pH and production of the organic acids which are competing for sorption sites and liberate Po by chelating Fe and Al oxides (Jones 1998). Excretion of organic acids, enzymes and protons by roots may play a major role in the P nutrition of various crops (Raghothama 1999; Uren and Reisenauer 1988). Rhizosphere phosphatases play an important role in the release of Pi from organic soil P, for subsequent uptake by plants. Enhancement of acid phosphatase activity with phosphate starvation has been demonstrated for maize (Helal and Sauerbeck 1987; Kummerova 1986).

Microbial mineralization/decomposition of the organic matter is necessary for nutrient cycling in soils and phosphatases augment the use of organically bound compounds by higher plants. Plants, particularly in nutrient-poor habitats like forest eco-system, mostly depend on symbiotic relations with microbes like mycorrhizal fungi. However, rhizosphere microbes might decompose P mobilizing substances derived from plant roots. Microbes can be strong competitors for growth limiting nutrients like P; however microbial turnover can make P available for higher plants. Acid phosphatases play a vital role in the mineralization of organic-P in soil. Soil microorganisms are known to enhance the availability of P for the growth of plants by solubilizing the insoluble forms of P in soil (Kucey et al. 1989). However, for plant grown in either neutral or acidic soils, P acquisition was dependent on extensive root exploitation and high phosphatase activity in the root zone especially alkaline phosphatase and diesterase, indicating microbial facilitation of organic P mineralization (Marschner et al. 2006).

Organic P may also make up a large fraction of soluble P, as much as 50% in soils with high organic matter content (Barber 1984). The organic P pool comes from microbial,
plant and animal residues deposited on or in the soil. Most of the organic pool is found in the top layers of soil and nearly half of this is in the form of phytic acid. Each molecule of phytic acid has the potential to release six molecules of orthophosphate (\(\text{H}_2\text{PO}_4^-\) or \(\text{HPO}_4^{2-}\)) to the soil solution. Phytate, a hexaphosphate salt of inositol, is the major form of P in organic matter, contributing between 50 and 80% of the total organic P (Alexander 1977). Although microorganisms are known to produce phytases that can hydrolyze phytate, phytate tends to accumulate in virgin soils because it is rendered insoluble as a result of forming complex molecules with Fe, Al and Ca (Alexander 1977). Phospholipids and nucleic acids form a mother pool of labile P in soil that is easily available to most of the organisms present (Molla and Chowdary 1984).

![Figure 1.2 Schematic diagram of soil phosphorus mobilization and immobilization by bacteria (Adapted from Mohammadi and Sohrabi 2012)](image)

### 1.2.3 Potassium

Potassium (K) is abundant (0.5–2.5%) in most soils but a small fraction of it is available to plants. Contrary to nitrogen and phosphorus, in most tropical soils, need for potassium arises because it becomes deficient due to leaching and/or fixation. The unavailable form of K in soil is about 90–98% of the total K in soil. About 0.1–2% of this amount is readily available. Further, unlike nitrogen and phosphorus, potassium forms no
coordinated compounds; rather, it exists solely as K⁺ either in soil solution or bound to negative charges on tissue surfaces through radicals (Maheshwari et al. 2012).

When potassium is limiting, the deficiency symptoms appear on the leaf surfaces in the form of white spots on their edges. Sometimes, chlorosis and necrosis of the leaf edges are also seen. The symptoms arise first on the lower leaves progressing toward the top as the severity of the deficiency increases. Potassium deficiency may also occur in young leaves at the top of high-yielding fast-maturing crops (Sperberg 1958). Potassium plays an important role in the growth and development of plants. It activates enzymes, maintains cell turgor, enhances photosynthesis, reduces respiration, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis. In addition to plant metabolism, potassium improves crop quality because it helps in grain filling and kernel weight strengthens straw, increases disease resistance and helps the plant better to withstand stress (Maheshwari et al. 2012).

Microorganisms play a key role in the natural K cycle. Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. There are considerable population of K solubilizing bacteria in soil and rhizosphere (Sperberg 1958). Silicate bacteria were found to dissolve potassium, silicon and aluminium from insoluble minerals (Aleksandrov et al. 1967). It has been reported that most of potassium in soil exists in the form of silicate minerals. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch et al. 1985). Mineral potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan et al. 1996). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, illite and orthoclases through production and excretion of organic acids as shown in Fig. 1.3 (Fridrich et al. 1991).
1.2.4 Sulphur

In Indian soil, sulphur (S) is abundant in fine-texture soil than in coarse soil because the latter contains a greater amount of organic matter. S is the fourth major nutrient besides nitrogen, phosphorus, and potassium because its availability to growing plants allows certain amino acids (cysteine and methionine) to be synthesized in optimum amounts. S deficiency was observed initially in the crops that were raised in dry land and in rice in wet land. The increased application of low S fertilizers and reduced use of organic manure resulted in S deficiency in Indian soil (Maheshwari et al. 2012).

Under the condition of S deficiency, protein synthesis is impaired and the level of chlorophyll in the photosynthetic organism declines. During the early phases of sulfur deficiency in leguminous plants, the rate of nitrogen fixations decline more than the rate of photosynthesis. Sulfur deficiency in plants usually appears on the leaves in the form of light green coloration, intervein chlorosis, stripes on upper leaves etc. (Maheshwari et al. 2012). These symptoms can be overcome by foliar application of elemental S in the form of dust. This normally alleviates S deficiency in the crop besides protecting it from rust disease. To overcome the issue of deficiency, S fertilizers are added mainly in reduced form such as elemental S. This form must be oxidized to sulfate by the
involvement of microorganisms. Plants can easily take up S in the form of sulfates. Sulfur occurs in soil in different forms mainly as elemental S, mineral sulfide, sulfates, hydrogen sulfide, organic S compounds etc. Inorganic form of S in soil occurs as sulfate and in anaerobic conditions, it is present in a reduced form (Kotková et al. 2008).

Sulfur interacts with phosphorus (P) available in soil and such process allows a greater absorption of \( \text{PO}_4^{3-} \) in comparison to that of \( \text{SO}_4^{2-} \) by growing crop plants. During this process, desorption of \( \text{SO}_4^{2-} \) also occurs due to substitution of \( \text{PO}_4^{3-} \) on the absorption site of \( \text{SO}_4^{2-} \). This may cause leaching of S in soil, resulting in S deficiency following application of phosphate. Lime (CaO) releases \( \text{SO}_4^{2-} \) in soil and makes the soil neutral, which is a desirable characteristic to neutralize acid soil. Conversely, application of elemental S to alkaline soil increases the availability of other nutrients such as Fe, Zn, and Mn. Various microbes utilize sulfur compounds as electron donors and oxidize them in the soluble form of sulfate during the biogeochemical cycle. A diverse range of bacteria of different physiological natures, viz. chemolithotrophs, photolithotrophs, photoheterotrophs etc. play different roles in S oxidation (Brüser et al. 2000).

1.2.5 Calcium and Magnesium

Calcium (Ca) is important for the stability and function of plant membranes and in case of Ca deficiency, there is membrane leakage of low-molecular-weight compounds, e.g., sugars and amino acids, from the cytoplasm to the apoplast, which stimulate infection by the pathogens (Marschner 1995). Further, Ca is an important component of the cell wall structure, as calcium polygalacturonates are required in the middle lamella for cell wall stability. When Ca concentration drops, there is an increased susceptibility to fungi which preferentially invade the xylem and dissolve the cell walls of the conducting vessels, which leads to wilting symptoms. In addition, plant tissues low in Ca is also much more susceptible than tissues with normal Ca levels to parasitic diseases during storage. It also plays a role in N metabolism as it enhances plant uptake of \( \text{NO}_3^- \). Other important functions of Ca include the movement of carbohydrates and other nutrients within the plant and cell elongation and division (Graham 1983).
Manganese (Mn) is probably the most studied micronutrient as regards its effects on disease and is important in the development of resistance in plants to both root and foliar diseases (Graham and Webb 1991; Huber and Graham 1999; Heckman et al. 2003). Mn availability in the soil varies and depends on many environmental and soil biotic factors. Mn is required in much higher concentration by higher plants than by fungi and bacteria and there is opportunity for the pathogen to exploit this difference in requirement (Marschner 1995). Magnesium is a critical component of chlorophyll, and therefore essential for photosynthesis in the plant. It acts as a catalyst and co-factor for many important enzyme systems within plants and also appears to play a role in the production of oils and fats.

1.2.6 Iron

Iron (Fe) is one of the most important micronutrients for animals and humans and the interaction between Fe nutrition and human or animal health has been well studied, as it is involved in the induction of anemia. Several plant pathogens, e.g., *Fusarium*, have higher requirements for Fe or higher utilization efficiency compared with higher plants. Therefore, Fe differs from the other micronutrients such as Mn, Cu, and B, for which microbes have lower requirements. Fe can control or reduce the severity of several diseases such as rust in wheat leaves, smut in wheat, and *Colletotrichum musae* in banana (Graham and Webb 1991; Graham 1983). Foliar application of Fe can increase the resistance of apple and pear to *Sphaeropsis malorum* and cabbage to *Olpidium brassicae* (Graham 1983).

Rhizosphere microorganisms can synthesize siderophores which can lower Fe level in the soils. Siderophore is low molecular weight compounds (400–1,500 Dalton) preferentially chelate iron (Fe\(^{3+}\)) and transport it into the cell across the cell membrane (Wandersman and Delepelaire 2004). The bacterium that originally synthesized the siderophores takes up the iron siderophore complex by using a receptor that is specific to the complex and is located in the outer cell membrane of the bacterium. Once inside the cell, the iron is released and is then available to support the microbial growth. Iron is an important micronutrient used by bacteria and it is essential for their metabolism. In the soil, it is
unavailable for direct assimilation by microorganisms because ferric iron (Fe$^{3+}$), which predominates in nature, is only sparingly soluble and too low in concentration to support microbial growth (Raehid and Ahmed 2005). To survive, soil microorganisms synthesize and secrete this low-molecular iron binding compound. The siderophores bind most of the Fe$^{3+}$ in the rhizosphere and effectively prevent the proliferation of fungal pathogens by depriving them of available iron (Kloepper et al. 1980). Suppression of the pathogens arises because iron deficiency causes growth inhibition, decrease in nucleic acid synthesis inhibition of sporulation and causes changes in cell morphology (Mathiyazhagan et al. 2004).

In earlier literature iron chelators like ferrichromes, ferrioxamines etc. were termed as sideramines and sideromycins which are today collectively called siderophores. These molecules are also known as ionophores. Generally they are categorized into two groups 1) Hydroxamates 2) Catecholates. Winkelman and Dreschel (1997) have classified bacterial siderophores into 5 types. 1) Catecholates 2) Hydroxamates 3) Peptide siderophores 4) Mycobactin and 5) Citrate hydroxamates. Hydroxamates contain three secondary hydroxamate group (C=O, N-(OH) and R where R is an amino acid chain or its derivative. Each hydroxamate group provided 2 oxygen atoms which form a bidentate ligand with iron. Therefore each siderophores forms a hexadentate octahedral complex with Fe$^{2+}$. Catecholates are chemically derivatives of 2, 3, dihydroxy benzoic acid. Each catecholate provided two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed (Chincholkar et al. 2000).

One of the suggested modes of growth promotion of nodulate legumes under field conditions is by microbial production of siderophores which facilitate the uptake of iron from the environment (Ahemad and Khan 2010). Specific siderophore producing Pseudomonas strains rapidly colonized roots of several crops and resulted in increased yield (Schroth and Hancock 1982). Enhanced plant growth caused by Pseudomonas strains was often accompanied by the reduction in pathogen populations on the roots. There is convincing evidence to support a direct role of siderophore mediated iron competition in the biocontrol activity exhibited by such isolates. The antagonism depends on the amount of iron available in the medium, siderophores produced by a biocontrol
agent and sensitivity of target pathogens. Siderophore-producing rhizobacteria improve plant health at various levels: they improve iron nutrition, inhibit growth of other microorganisms with release of their antibiotic molecule and hinder the growth of pathogens by limiting the iron available for the pathogen, generally fungi, which are unable to absorb the iron siderophore complex.

Figure 1.4 Iron acquisition mechanisms (Adapted from McLaughlin et al. 2011)

Microbes possess at least 4 mechanisms that could mediate iron acquisition: (i) acquisition of protein-bound iron (ii) extracellular and/or surface-bound iron reductases (iii) a citrate inducible ferric citrate uptake system and (iv) siderophore and siderophore-like uptake systems. Experimentally verified iron acquisition systems include HupDGC, involved in uptake of hemin and hemoglobin, and Fhu; involved in uptake of ferrichrome siderophores. Within the cell, iron can be stored bound to the Fri protein. The Fur regulator binds to the Fur box in the presence of iron and is released in low iron conditions. Mechanism (i) is likely to play a more significant role inside rather than
outside the host, whereas mechanisms (ii, iii, and iv) are likely to play a significant role both inside and outside of the host as shown in Fig. 1.4.

1.2.7 Zinc

Although, Indian soil contains adequate amount of Zinc (Zn), it is inadequate to meet the requirements of the crop plants (Singh 2001). Therefore, the role of bacteria lies in solubilizing insoluble source of zinc compounds into more accessible forms similar to the phosphate-solubilization process involving certain phosphate-solubilizing microorganisms. Zinc plays an important role in protein and starch synthesis, and therefore a low zinc concentration induces accumulation of amino acids and reducing sugars in plant tissue (Marschner 1995; Romheld and Marschner 1991). As an activator of Cu/Zn-SOD, Zn is involved in membrane protection against oxidative damage through the detoxification of superoxide radicals (Cakmak 2000). Application of Zn to the soil reduced infections by Fusarium graminearum (Schwabe) and root rot diseases, caused by Gaeumannomyces graminis (Sacc.) in wheat (Graham and Webb 1991; Grewal et al. 1996).

Zinc-deficiency symptoms such as chlorosis of leaves occur due to its nonavailability and alterations. The most common feature of zinc deficiency is stunted growth and smaller leaves on account of disturbance in the auxin and indole-3-acetic acid metabolism, an indicator of Zn deficiency in the plant tissues. Baruah and Barthakur (1999) observed that Zn solubility by certain groups of microorganisms increased corresponding to decrease in pH. This kind of reaction mechanism involved the action of micromediated organic acid production responsible for Zn solubilization. The Zn-solubilizing bacteria confer certain mechanism-transforming insoluble forms of Zn into solubilized forms that could easily be taken up by plants. In such cases, the PGPR group of bacteria, in particular Bacillus spp. and fluorescent Pseudomonas, showed Zn solubilization of a wide range of Zn compounds. On the other hand, Azotobacter chroococcum, Bacillus megaterium, and B. edaphicus increased the availability of Zn in the soil, but molecular mechanisms of Zn-solubilization process are still a subject of research interest for microbiologists.
1.2.8 Other Micronutrients

Although micronutrients (Cl, Mn, B, Cu, and Mo) are required in “micro” quantities, they are significant in terms of their contribution to plant growth. Soil characteristics can influence micronutrient availability. Clay soils are less likely to be deficient in micronutrients than sandy soils. Soils with low (i.e., less than 1 to 2%) or very high organic matter content (i.e., greater than 30%) often have low levels of micronutrient availability. As soil pH increases, availability of micronutrients tends to decrease. The exception is molybdenum whose availability increases with soil pH. Inorganic micronutrients occur naturally in mineral soils. As parent minerals break down during soil formation, micronutrients slowly become available to plants. Organic matter is also an important source of micronutrients. Microbial decomposition helps to release micronutrients into plant-available forms (Table 1.1).

1.3 Other plant growth promoting traits

1.3.1 Indole acetic acid (IAA)

PGPR produce phytohormones that are believed to be related to their ability to stimulate plant growth. Indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement (Salisbury 1994). This hormone is very commonly produced by PGPR. Most commonly, IAA-producing PGPR are believed to increase root growth and root length, resulting in greater root surface area which enables the plant to access more nutrients from soil. These rhizobacteria synthesize IAA from tryptophan by different pathways as shown in Fig. 1.5, although it can also be synthesized via tryptophan-independent pathways, though in lower quantities (Spaepen et al. 2007). Phytopathogenic bacteria mainly use the indole acetamide pathway to synthesize IAA, which has been implicated in tumor induction in plants. In contrast, the indole pyruvic acid pathway appears to be the main pathway present in plant growth promoting beneficial bacteria (Patten and Glick 2002). Auxins synthesized by the plant and the microorganisms differ only in the biosynthetic pathway, depending on the plant and/or microorganism.
Introduction

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>From taken up by plants</th>
<th>Function in plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorine (Cl)</td>
<td>Cl(^{-}) ion</td>
<td>Associated with the suppression of leaf and root diseases. It is essential in photosynthesis.</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>Fe(^{2+})/Fe(^{3+}) ion</td>
<td>A vital constituent of chlorophyll. Important for oxygen transfer within the plant system. Fe has been strongly associated with protein metabolism.</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>Mn(^{2+}) and as a component of organic complexes</td>
<td>Seems to play a role in the uptake of other nutrients and the activation of a number of enzymes systems.</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>Zn(^{2+}) and as a component of organic complexes</td>
<td>Plays a role in the formation of growth promoting compounds, carbohydrates transformation, regulation of sugar consumption, and is a constituent of several enzyme systems. Zn has a role in RNA and protein synthesis.</td>
</tr>
<tr>
<td>Boron (B)</td>
<td>Primarily boric acid (H(_3)BO(_4))</td>
<td>Maintains plant cell wall integrity. It shown to promote root growth.</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>Cu(^{2+}) and as a component of organic complexes</td>
<td>Essential for production of Fe-containing compounds. Facilitates synthesis of chlorophyll. Influences several metabolic reactions.</td>
</tr>
<tr>
<td>Molybdenum (Mo)</td>
<td>MoO(_4^{2-})</td>
<td>Enhance plant uptake of N, K and Ca required by legumes for fixation of atmospheric N by nodular bacteria. Assists in making Fe plant available.</td>
</tr>
</tbody>
</table>

Table 1.1 Function of micronutrients in plants (Modified and adapted from Uchida 2000)
Interactions between IAA-producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phytostimulation. Reviewing the role of bacterial IAA in different microorganism–plant interactions highlights the fact that bacteria use this phytohormone to interact with plants as part of their colonization strategy, including phytostimulation and circumvention of basal plant defense mechanisms. Moreover, several recent reports indicate that IAA can also be a signaling molecule in bacteria and therefore, can have a direct effect on bacterial physiology (Spaepen et al. 2007). There are numerous soil microflora involved in the synthesis of auxins in pure culture and soil (Barazani and Friedman 1999). IAA released by rhizobacteria mainly affects the root system, increasing its size and weight, branching number and the surface area in contact with soil. All these changes lead to an increase in its ability to probe the soil for nutrient exchange, therefore improving plant nutrition and growth capacity (Gutiérrez Mañero et al. 1996). Another important result of inoculation with auxin-producing bacteria is the formation of adventitious roots, which derive from the stem. The auxins induce the stem tissues to redifferentiate as root tissue. All the above effects can vary considerably depending on the auxin concentration that reaches the root system, including an excess that could be inhibitory.

![Biosynthetic pathway of IAA synthesis in bacteria](image-url)

**Figure 1.5** Biosynthetic pathway of IAA synthesis in bacteria (Adapted from Ahemad and Khan 2011)
1.3.2 Ammonia Production

Ammonia production is related with the nitrogen fixation and mostly observed in the leguminous rhizobacteria. However, during this study bacteria isolated from the rhizosphere of Jatropha curcas showed the production of ammonia having the indication of nitrogen fixation. Important biochemical reactions of biological nitrogen fixation occur mainly through symbiotic association of nitrogen fixing microorganisms with legumes that converts atmospheric elemental nitrogen (N₂) into ammonia (NH₃) (Shiferaw et al. 2004). A range of plant growth promoting rhizobacteria participate in interaction with C3 and C4 plants (e.g. rice, wheat, maize, sugarcane, jatropha and cotton), and significantly increase their vegetative growth and grain yield (Kennedy et al. 2004). Azospirillum species are aerobic heterotrophs that fix N₂ under microaerobic conditions and grow extensively in the rhizosphere of gramineous plants (Kennedy et al. 2004). ¹⁵N tracer techniques showed that Azospirillum brasilense and Azospirillum lipoferum contributed 7–12 % of wheat plant N by biological nitrogen fixation (Malik et al. 2002). Inoculation with Azospirillum brasilense significantly increases N contents of cotton up to 0.91 mg plant⁻¹ (Fayez and Daw 1987). Inoculation with Azospirillum also significantly increased N content of sugarcane leaves in greenhouse experiments (Muthukumarasamy et al. 1999) which reflects the production of ammonia by Azospirillum.

1.3.3 Exopolysaccharide production

Some plant growth-promoting strains can produce bacterial exopolysaccharide (EPS). They can remain associated with the cell wall to form a bound capsule layer or they can be released in to cells surrounding as extracellular slime (Glick et al. 1999). EPS have vital roles in a variety of processes such as formation of biofilm (Bhaskar and Bhosle 2005), protection of bacterial cell from desiccation (Pal et al. 1999), for maintaining primary cellular functions and antibacterial activity against predators, gelling ability, pollutant degradation kinetics (Fusconi and Godinho 2002), bioremediation activity and plasma substituting capacity (Allison 1998). Recent literature reports encouraging results about the potential use of microbial EPS as antitumor and immune-modulator agents (Xu et al. 2009). EPS-producing PGPR can significantly enhance the volume of soil macropores and the rhizosphere soil aggregation, resulting in increased water and
fertilizer availability to inoculated plants. EPS production is reported in strains like *Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus mutans* (Vimala and Lalithakumari 2003). EPS-producing PGPR can also bind cations including Na⁺. Therefore, an increase in the population density of EPS-producing bacteria in the root zone is expected to decrease the content of Na⁺ available for plant uptake, and thereby alleviate salt stress in plants growing in saline environments. These polysaccharides play important roles in many biological processes, and they can function as the virulence determinants in the pathogens (Peng et al. 2008). The production of EPS is a very common trait among bacteria and is probably a critical determinant for achieving successful colonization of any surface. In addition exopolysaccharides may be involved in cell aggregation and their synthesis may increase the chances of survival for the bacteria under desiccation or nutrient deprived conditions and helps in nitrogen fixation by preventing high oxygen tension (Glick et al. 1999).

### 1.3.4 HCN production

HCN is a volatile, secondary metabolite that suppresses the development of microorganisms (Siddiqui et al. 2006). HCN is a powerful inhibitor of many metal enzymes, especially copper containing cytochrome C oxidases. HCN is formed from glycine through the action of HCN synthetase enzyme, which is associated with the plasma membrane of certain rhizobacteria (Blumer and Haas 2000). To date many different bacterial genera have shown to be capable of producing HCN, including species of *Alcaligenes, Aeromonas, Bacillus, Pseudomonas* and *Rhizobium* (Devi et al. 2007; Ahmad et al. 2008). Cyanide is a phytotoxic agent capable of inhibiting enzymes involved in major metabolic processes and is considered one of the typical features of deleterious rhizobacterial isolates (Bakker and Schippers 1987). Nevertheless, at present its applications in areas of biocontrol methods are increasing (Devi et al. 2007). Some cyanogenic rhizobacteria are typically host specific and associated with the roots of their host plants. Therefore, HCN produced in the rhizosphere of seedlings by selected rhizobacteria is a potential and environmentally compatible mechanism for biologically controlling weeds and minimizing deleterious effects on the growth of desired plants (Kremer and Souissi 2001).
1.4 Co-inoculation of rhizobacteria for integrated nutrient management

Recently, there has been a shift in approach of workers, as instead of using single strain of plant growth promoting rhizobacterium as inoculant, nowadays co-inoculation of two or multiple PGPR is experimented to achieve prominent multifarious effect on productivity for improving sustainable agriculture system. Seneviratne (2003) recognized that co-inoculation and co-culture of microbes perform the task better than the individual microbes. However, in recent years, many studies have shown that co-inoculation of rhizobia and some plant growth promoting bacteria (PGPB) increases nodulation and growth in a wide variety of legumes (Bullied et al. 2002; Shaharoona et al. 2006, Tilak et al. 2006). Earlier, microbial studied performed without plants indicated that some combinations allow the bacteria to interact with each other synergistically, provide nutrients, remove inhibitory products and stimulate each other through physical and biochemical activities that may enhances some beneficial aspects of their physiology (Bashan 1998). When the two different strains are made into an inoculum consortium, each of the individual strains of the consortium not only outcompetes with each other for rhizospheric establishment but also complements functionally for plant growth promotion (Shenoy and Kalagudi 2003). Combined use of PGPR is based on the principals of natural ecosystems, sustained by their constituents. In other terms, the quantity and quality of inhabitants and specific ecological parameters i.e., the greater the diversity and the number of inhabitants, the higher the order of their interaction and more stable the ecosystem. This concept of combined use of PGPR is an effort to shift microbiological equilibrium in favour of increased plant growth production, nutrient uptake and protection (Higa 1991; Parr et al. 1994).

Gupta et al. (2002) reported results for the management of fungal diseases by using microbial combination involving S. fredii KCC5 and P. fluorescens LPK2 reduced wilt disease, proved the most effective in reducing disease incidence. Co-inoculation with Azospirillum (Amutha et al. 2009) is based on mixed inoculants, combinations of microorganisms that interact synergistically, where Azospirillum function as a "helper" bacteria, which enhance the performance of other beneficial microorganisms. On the other hand it has been found that these bacteria would also interact synergistically by
providing nutrients, removing some inhibitory products, or stimulating each other through physical or biochemical mechanisms (Khammas and Kaiser 1992). Co-inoculation of legumes with symbiotic and free living microbes like *Azotobacter*, *Azospirillum* and *Acetobacter* has received great attention in recent years (Lakshmann 2000). Free-living diazotrophs increase the lateral roots and root hair density resulting in more infection sites for rhizobia, thus enhancing the N₂-fixing ability of legumes (Parmar and Dadarwal 1999). In bean plants, nodulation was increased when *Rhizobium* spp. were co-inoculated with *Bacillus polymyxa* (Petersen et al. 1996) or *Azospirillum brasilense* (Burdman et al. 1996). In soybean plants, synergism between *Bacillus* and *Rhizobium* in the rhizosphere promotes plant growth and development (Halverson and Handelsman 1991). Inhibition of nodulation by rhizobacteria has also been reported (Plazinski and Rolfe 1984).

1.5 Influence of Mycorrhizal Fungi on integrated nutrient management

Mycorrhizal symbioses are found in almost all ecosystems and can enhance plant growth through a number of processes which include improvement of plant establishment, increased nutrient uptake (particularly P and essential micronutrients such as Zn and Cu, but also N and, depending on soil pH, may enhance the uptake of K, Ca and Mg; Clark and Zeto 2000), protection against biotic and abiotic stresses and improved soil structure (Buscot 2005; Smith and Read 2008). Mycorrhizal fungi typically colonize the root cortex biotrophically and develop external hyphae (or extra-radical mycelia) which connect the root with the surrounding soil. The establishment of mycorrhizal fungi in roots changes key aspects of plant physiology, including mineral nutrient composition in tissues, plant hormonal balance and patterns of carbon allocation. The fungi may also alter the chemical composition of root exudates, whilst the development of mycelium in soil can act as a carbon source for microbial communities and introduce physical modifications to the soil environment (Gryndler 2000).

The fungi enhance immobile nutrient uptake by increasing the absorptive surfaces of the root. The supply of immobile nutrients to roots is largely determined by the rate of diffusion. In soils not adequately supplied with nutrients, uptake of nutrients by plants far
exceeds the rate at which the nutrients diffuse into the root zone, resulting in a zone around the roots depleted of the nutrients. Mycorrhizal fungi help to overcome this problem by extending their external hyphae to areas of soil beyond the depletion zone, thereby exploring a greater volume of the soil than is accessible to the unaided root. Mycorrhizal fungi may have biochemical capabilities for increasing the supply of available phosphorus and other immobile nutrients. These capabilities may involve increases in root phosphatase activity, excretion of chelating agents, and rhizosphere acidification (Habte 2000). Mycorrhizal fungi are often implicated in functions which may or may not be related to enhance nutrient uptake. For example, they have been associated with enhanced chlorophyll levels in leaves and improved plant tolerance of diseases, parasites, water stress, salinity, and heavy metal toxicity (Bethlenfalvay 1992). Moreover, there is increasing evidence that hyphal networks of mycorrhizal fungi contribute significantly to the development of soil aggregates, and hence to soil conservation (Miller and Jastrow 1992).

Bacteria associated to mycorrhizal fungi adhere to fungal spores and hyphal structures and thus spread to the rhizosphere (Bianciotto and Bonfante, 2002). Bianciotto et al. (2004) observed strong evidence of a vertical transmission of endobacteria through the mycorrhizal vegetative generation. However, antagonistic effects are often reported in the mycorrhiza-PGPR interactions. Positive interactions often result in plant growth improvement. A typical beneficial effect is that exerted by ‘mycorrhizal-helper-bacteria’ (MHB) which stimulate mycelial growth and/or enhance mycorrhizal formation (Garbaye 1994). Responses to MHB are associated with both the production of compounds that increase root cell permeability and rates of root exudation, which either stimulate fungal mycelia in the rhizosphere or facilitate root penetration by the fungus, and the production of phytohormones that influence mycorrhiza establishment (Barea et al. 2005). Specific rhizobacteria are also known to affect the pre-symbiotic stages of mycorrhizal fungi development, such as spore germination and rate of mycelial growth (Barea et al. 2005). Recently, Frey-Klett et al. (2007) revisited the significance of MHB and differentiated the effects based on either mycorrhizal fungi formation or mycorrhizal fungi function,
including nutrient mobilization, N₂ fixation and protection of plants against root pathogens.

1.6 Influence of different soil amendments on integrated nutrient management

1.6.1 Vermicompost

Vermicompost is one of the best processes of recycling of different types of wastes available on farm and may become most important components of integrated nutrient management (INM) systems. Since it is a natural organic product which is ecofriendly, it does not leave any adverse effects either in soil or in the environment. Thus, much interest in vermicomposting has been generated due to the fact that earthworms play an important role in soil improvement, organic matter decomposition and enhancing plant growth (Gupta and Bhagat 2004). On an average vermicompost contains 1.6% N, 5.04% P₂O₅ and 0.8% K₂O. There are also good amount of calcium (Ca), magnesium (Mg), zinc (Zn) and manganese (Mn). Apart from this it also contains hormones like auxins and cytokinins, enzymes, vitamins and useful microorganisms like bacteria, actinomycetes, protozoa, fungi and others. Annual application of adequate amount of vermicompost also lead to significant increase in soil enzyme activities such as ‘urease’, ‘phosphomonoesterase’, ‘phosphodiesterase’ and ‘arylsulphatase’. The soil treated with vermicompost has significantly more electrical conductivity (EC) and near neutral pH. Vermicompost has very high porosity, aeration, drainage and water holding capacity (Tiwari et al. 1989). They have a vast surface area, providing strong absorbability and retention of nutrients. They appear to retain more nutrients for longer period of time. Study showed that soil amended with vermicompost had significantly greater soil bulk density and hence porous and lighter and never compacted. Positive effects of vermicompost include stimulated seed germination in several plant species such as green gram (Lazcano et al. 2010). Vermicompost also has a positive effect on vegetative growth, stimulating shoot and root development (Edwards et al. 2004). The effects include alterations in seedling morphology such as increased leaf area and root branching (Lazcano et al. 2009). Vermicompost has also been shown to stimulate plant flowering, increasing the number and biomass of the flowers produced (Atiyeh et al. 2002; Arancon et al. 2008), as well as increasing fruit yield (Atiyeh, et al. 2000; Arancon et al. 2004a,
In addition to increasing plant growth and productivity, vermicompost may also increase the nutritional quality of crops (Lazcano et al. 2011).

1.6.2 Humic acid

Humic acids comprise a set of heterogeneous organic molecules, self-assembled in organic aggregates, stabilized by H bonding and hydrophobic interactions, present in soil, water, sediments, and organic residues (Piccolo 2002). It was previously observed that humic acids can act as plant growth promoters, particularly in the root system (Façanha et al. 2002; Zandonadi et al. 2007) and nutrient uptake (Chen et al. 2004). Humic acid contains growth promoting substances and indirectly helps in promoting growth and yield of crops by decreasing IAA oxidase activity and promoting metabolic activities consequently accelerates growth and yield of crops. Humic acid enhanced plant growth by increasing the adsorption of ions facilitating chelation of micronutrients and also contains growth promoters. The major advantages of humic acid in agriculture are, it plays a vital role in enhancing the nutrient uptake of crop, prevents losses of degradation and leaching of nutrients, improves the water retention capacity, porosity, aggregates stability, cation exchange capacity, reduces the use of inorganic fertilizers, helps in building up of organic matter and favourable microbial population of the soil. It develops resistance to the plants through reduced amino acid synthesis by diverting free amino acid to protein, synthesis of phenols and indole compounds from the aromatic amino acids, enhanced the levels of organic matter solubilizes silica associated with higher concentration of silica in plants improving plant resistance to pest and disease (Kowalski and Davies 1982).

It could be inferred that humic acid application along with recommended inorganic fertilizers and organic manures plays a greater role in plant bio-chemical and physiological activities and soil fertility, consequently resulting in better growth and yield of crops. On the other hand, if humic acid was applied at higher level it hampers the growth and yield of crops.
1.7 Salinity stress

For centuries, agriculture in arid and semiarid environments has faced an increase in soil salinity. Salinity is one of the major abiotic stress factor limiting plant growth and productivity (Khan and Panda 2008). Globally, some 20% of irrigated land (450,000 km²) is salt affected, with 2,500–5,000 km² of production lost every year as a result of salinity (UNEP 2009). Therefore, with increasing population and rise in food demand, the utilization of salt-affected soils for agriculture will become necessary to feed the growing population of the world. Soil salinity is defined as the concentration of dissolvable salts extracted from soil by water (Richards 1954). Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in the areas devoted to agriculture; therefore, the urgent need of biological agents (biopreparations) is accepted worldwide. For this, evolving low cost, easily adaptable technologies become essential and are now considered as a major challenge.

Salt stress affects many aspects of plant metabolism and as a result, growth and yield are reduced. Excess salt in the soil solution may adversely affect plant growth either through osmotic inhibition of water uptake by roots or specific ion effects. Specific ion effects may cause direct toxicity or, alternatively, the insolubility or competitive absorption of ions may affect the plant’s nutritional balance. Salinity was shown to increase the uptake of Na⁺ or decrease the uptake of Ca²⁺ and K⁺ (Yildirim et al. 2006). Accumulation of excess Na⁺ may cause metabolic disturbances in processes where low Na⁺ and high K⁻ or Ca²⁻ are required for optimum function. Uptake and accumulation of Cl⁻ may disrupt photosynthetic function through the inhibition of nitrate reductase activity (Xu et al. 2002). Soil salinity affects plants in three ways. The primary effect of increased salt concentration in the soil is the inability of plants to draw water from the soil, even though the soil appears quite moist. In effect, the plant suffers from a form of drought which can result in retarded growth and reduced yield. Secondly, some salts, such as Na and Cl enter the plant system through water and affect the plant’s physiological processes often resulting in reduced growth, leaf burn and even plant death. Thirdly, high amounts of
ions such as Na and Cl affect the availability of other essential plant nutrients like K, Mg, N, or P which are extremely important for plant growth (Tester and Davenport 2003).

Salt resistant plants are categorized into two groups: salt-excluders and salt-includers. The former group of plants adapt to a saline environment by avoiding salt, whereas the includers take up salt and sequester it. Biochemical strategies to cope with salt stress includes: 1) Selective accumulation or exclusion of ions 2) Control of ion uptake by roots and transport into leaves 3) Compartmentalization of ions at the cellular and whole-plant levels 4) Synthesis of compatible solutes 5) Alteration of membrane structure 6) Induction of ant oxidative enzymes and 7) Induction of plant hormones (Parida and Das 2005). This has been shown by Arora et al. (2012) in Fig. 1.6.

In addition to the use of traditional breeding and transgenic plants, the utilization of plant growth promoting rhizobacteria (PGPR) is useful in strategies to facilitate plant growth in saline soils (Tank and Saraf 2010). Many PGPR facilitate plant growth indirectly by reducing plant pathogens or directly by facilitating the uptake of nutrients from environment. It has also been observe that plants inoculated with PGPR having different PGPR traits are more resistant to deleterious effect of stress ethylene synthesized as a consequence of stress conditions (Zahir et al. 2008). In addition to facilitating plant growth, PGPR can protect plants from deleterious effects of environmental stresses including flooding, drought, salinity and phytopathogens (Yildrim et al. 2006). PGPR tested on growth of pepper, tomato, bean, canola and lettuce under salt stress ameliorated deleterious effects of salinity (Glick et al. 1997). Reports also showed enhancement of squash plant when applied directly or as transplant under salinity stress (Yildrim et al. 2005). Microbial associations also provide host plants with required plant growth regulators and minerals like N, P and K even under stress conditions like salinity and drought (Vivas et al. 2003). Application of *Azotobacter* and *Flavobacter* have also resulted in to enhance uptake of P, Ca, and chloride and decrease Pb content in barley plants under field conditions (Han and Lee, 2005). Inoculation of plant with ACC deaminase containing PGPR has also resulted in to enhance chlorophyll contents of maize as well as lettuce (Glick et al. 1997; Han and Lee 2005). Mayak et al. (2004) also
evaluated the potential of rhizobacteria populating in dry salt environment to generate resistance in plants against induced salt stress.

1.7.1 Biochemical indicators of salinity tolerance in plants

The cytoplasmic membrane of bacteria is permeable to water but forms an effective barrier for most solutes in the medium and metabolites in the cytoplasm. To survive under osmotic stresses, the cells need to adapt by accumulating specific solutes under hyperosmotic conditions and releasing them under hypoosmotic conditions. Such solutes include K⁻, amino acids (e.g., glutamate and proline), amino acid derivatives (peptides and N-acetylated amino acids), quaternary amines (e.g., glycine, betaine, and carnitine), sugars (e.g., sucrose and trehalose) and tetrahydropyrimidines (ectoines) (Galinski and Truper 1994). These solutes are often referred to as compatible solutes because they can be accumulated to high levels by de novo synthesis or transported without interference with vital cellular processes. In fact, many compatible solutes proved to be effective stabilizers of enzymes, providing protection not only against high salt but also against high temperature, freeze–thawing and drying (Yancey et al. 1982). The bacterial cells may synthesize (some of the) compatible solutes following an osmotic upshock and degrade them following an osmotic downshift, but the initial response is much more rapid if compatible solutes can be taken up from the medium and/or released into the medium via semiconstitutive transport systems.

During saline conditions, generation of ROS such as the superoxide radical (O₂⁻), hydroxyl radical (OH), and hydrogen peroxide (H₂O₂) alter antioxidant activity. ROS causes oxidative damage to biomolecules such as lipids and proteins and eventually leads to plant death (Del Rio et al. 2003). PGPR such as S. proteamaculans and Rhizobium leguminosarum produce antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX), and catalase (CAT) and nonenzymatic antioxidants such as ascorbate, glutathione, and tocopherol. Ascorbate peroxidase (APX) is part of the scavenging cycle and catalyzes the reaction of ascorbic acid with H₂O₂, while glutathione reductase (GR) catalyzes the regeneration of ascorbic acid (Han and Lee 2005). To understand the protective action of antioxidants against salinity stress, plants were treated with PGPR
strains followed by measurement of the level of antioxidant activity. Inoculation with PGPR strains under salinity stress decreased enzyme activity with increasing salinity stress. Ruiz-Lozano et al. (2001) reported that mycorrhizal lettuce plants showed increased SOD activity under drought stress and this was correlated to plant protection against drought. Stress resistance in plants has been related to more effective antioxidant systems (Bor et al. 2003).

Figure 1.6 Increase in salt tolerance and survival of plant in saline habitat elicited by plant growth promoting rhizobacteria (Adapted from Arora et al. 2012)
1.7.2 Proteomic approach to salinity stress

Proteomics are valuable tool in delineation of different mechanism of stress responses in microbes. Proteome studies are important to get a clearer picture as proteins are the key functional units involved in physiological stress responses (Soni et al. 2011). Proteomic analysis is concerned with global changes in protein expression and typically involves a combination of 2D-PAGE for protein separation and visualization and mass spectrometry (MS) for protein identification. Proteomic analysis has contributed greatly to our understanding of gene function in the post-genomic era through protein chemistry. Two dimensional gel electrophoresis databases are the core of the bioinformatics for proteome research (Jang et al. 2006). There are large numbers of specific proteins reported in various genera of PGPR that showed increase in their level of expression upon adverse conditions such as salt. Due to its high-resolution, two-dimensional PAGE, combined with high-throughput mass spectrometry and bioinformatics, it is widely used for protein separation and identification, which is considered sufficiently discriminating to allow the unique identification of unknown proteins (Shen et al. 2002). Identification of differently displayed proteins could be used to ascertain the genes responding to relative physiological actions and clarify the functions of genes.

A proteomic study was also conducted in leaves to identify the mechanisms of salt responsiveness. These studies suggested the usefulness of proteomic approach in identifying functional proteins responsive to salt stress alone or in combination with other abiotic stress in plants. The protein identify in response to salt stress by proteomic approach are implicated in diverse physiological and defence processes. While some are probably part of the general stress response to help the plant to survive in stress conditions others may contribute to the negative physiological effects of salt (Die Lajudie et al. 1998).

1.8 Selection of Plant: Jatropha curcas L.

The depletion of world petroleum reserves and the increased environmental concerns have stimulated the search for alternative sources for petroleum-based fuel, including diesel fuels. Because of the closer properties, biodiesel fuel (fatty acid methyl ester) from
vegetable oil is considered as the best candidate for diesel fuel substitute in diesel engines. Biodiesel extracted from vegetable oil is one such renewable alternative under consideration. The production of biodiesel would be cheap as it could be extracted from nonedible oil sources. *Jatropha curcas* (Linaeus), a non-edible oil-bearing and drought-hardy shrub with ecological advantages, belonging to the *Euphorbiaceae* family, was found to be the most appropriate renewable alternative source of biodiesel. The *Jatropha* plant is a small tree or large shrub which can reach a height of up to 6 m. The branches contain latex. Normally, five roots are formed from seeds, one central (tap root) and four peripheral. The plant is monoecious and flowers are unisexual. Pollination is by insects. The life-span of the *Jatropha curcas* plant is more than 50 years (Henning 2004).

The best soils for *Jatropha* are aerated sands and loams of at least 45 cm depth (Gour 2006). Heavy clay soils are less suitable and should be avoided, particularly where drainage is impaired, as *Jatropha* is intolerant of waterlogged conditions. Ability to grow in alkaline soils has been widely reported, but the soil pH should be within 6.0 to 8.0/8.5 (FACT 2007). *Jatropha curcas* is also well adapted to gravelly, sandy and saline soil where salinity might be major problem due to limited water supply (Openshaw 2000). Gao et al. (2008) presented a result which indicates that *Jatropha curcas* L. seedling can tolerate NaCl concentration up to 150 mM and higher concentration can cause toxicity to plants. *Jatropha* can be grown over a wide range of arid or semi-arid climate conditions. For the emergence of seeds, hot and humid climate is preferred. Therefore, fairly warm summers with rains are beneficial for proper germination of seeds. The flowering is inducing in rainy season with reduction in temperature and plants bear fruits in winter. *Jatropha* can be cultivated with success in areas with scanty to heavy rainfall (Gubitz et al. 1999). *Jatropha* has been seen to be intolerant of frost. The plant is well adapted to conditions of high light intensity and is unsuited to growing in shade.

*Jatropha* is planted at densities ranging from 1100 to 2500 plants per ha. Yield per tree is likely to increase with wider spacing but with a decline in yield per ha (Achten 2008). Spacing decisions should be based on the environment, i.e. how it affects competition among trees for water, light and nutrients. Semi-arid, low-input systems should use wider
spacing such as 3.0 x 2.0, 3.0 x 2.5 or 3.0 x 3.0 meters. Alternate planting in succeeding rows will minimize mutual shading. In addition, consideration should be given to access. At least 2.5 m between trees allows easier passage for fruit pickers, while a 5-metre alley at every fourth row facilitates access by carts. Planting holes of 30–45 cm wide and deep should be prepared and organic matter incorporated before planting. The seedlings may require irrigation for the first two to three months after planting. Where *Jatropha* is being planted as a living hedge, cuttings of 60–120 cm length should be inserted between 5 and 25 cm apart and 20 cm into the ground. This should be done two to three months before the onset of the rainy season.

### 1.8.1 Application of *Jatropha curcas*

*Jatropha* plant has been found for using on different aspects in different communities in the world. On the basis of different literatures, some of the economic activities through the use of *Jatropha* can be broadly classified as follows:

**Potential as an oil crop**

Gubitz et al. (1999) reported that analysis of *Jatropha curcas* seeds shows that it contains: moisture 6.62; protein 18.2; fat 38.0; carbohydrates 17.30; fibre 15.50; and ash 4.5%. The oil content is 35 to 40% in the seeds and 50 to 60% in the kernel. The oil contains 21% saturated fatty acids and 79% unsaturated fatty acids. It has also been found that there are some chemicals element in the seeds which possess poisonous and purgative properties and render the oil non edible for human consumption.

**Industrial use**

*Jatropha* oil has very high saponification value and being extensively used for making soap in India and other countries. At present *Jatropha curcas* oil is being imported to meet the demand of cosmetic industry. The protein content *Jatropha* oil cake may be used as raw material for plastics and synthetics fibres. It would also be advantageous to make use of *Jatropha* oil as hydraulic oil (Gubitz et al. 1999).
Introduction

Medicinal use
The latex of *Jatropha curcas* contains an alkaloid known as Jatrophine which is believed to be having anti-cancerous properties. Curcas oil is used as an external application for skin diseases and rheumatism, it is reported to be abortifacent and also efficacious in dropsy, sciatic and paralysis. The leaf juice is used as an external application for piles. It is also applied for inflammations of the tongue in babies. The twig sap is considered stypic and used for dressing wounds and ulcers. The root is reported to contain yellow oil with strong anthelmintic action. The root bank is used to external application for sores. A decoction of the bark is given for rheumatism and leprosy.

Potential for enrichment of soil
*Jatropha* oil cake is rich in nitrogen, phosphorous and potassium and can be used as organic manure. Tender branches and leaves are also used as manure for coconut trees. *Jatropha* oil cakes can, hopefully, replace synthetic fertilizers by undertaking plantations of *Jatropha curcas* on wastelands, *Jatropha curcas* leaves provide plentiful organic matter and increase the microbial activity including earthworms which is an indication of ecological improvement of site (Gubitz et al. 1999).

Potential as a feed stock
*Jatropha* leaves are used as fed for the tusser silk worm. The oil cake is rich in protein but contains some toxic principle and as such it is considered unfit for use as cattle feed. But it is reported that the poisonous principle appears to exist in the alcohol soluble fraction of the oil. With suitable research it could be possible to convert the nonedible oil-cake into protein rich cattle and poultry feed on a massive scale (Gubitz et al. 1999).

Potential as insecticide/pesticide
The seeds are considered anthelmintic in Brazil. They are ground with palm oil and used as rat poison in Gabon. Aqueous extract to leaves is reported to have insecticidal properties. In Ghana, the leaves are for fumigating houses against bed bugs. The ether extract shows antibiotic activity against *Staphylococcus aureus* and *Escherichia coli*. The juice of the whole plant is used for stupefying fish (Gubitz et al. 1999).
Potential as profitable agro forestry crop
Owing to its multiple uses, there exists unlimited potential for extensive and convenient marketability of *Jatropha* oil, for indigenous as well as foreign markets. Simple and cost effective technology of growing *Jatropha* with or without irrigation makes it promising and profitable agro forestry crop ensuring optimal utilization of land, man power, water and financial resources. It is a crop with low capital investment, providing nonconventional energy in a decentralized manner and above all having a potential for wastelands development (Gubitz et al. 1999).

Potential as non-conventional energy crop
*Jatropha* oil is an environmentally safe, cost effective and renewable source of non-conventional energy as a promising substitute to Hydelpower, diesel, kerosene, LPG, coal and firewood etc. The fuel properties of the *Jatropha* oil closely resembles with the diesel oil. It was found that the specific gravity of *Jatropha* oil is 0.9180 (gr/ml) compared to diesel oil 0.8410 (gr/ml). Calorific value of the *Jatropha* oil is 41 MJ/kg and diesel oil is 45 MJ/kg (Rosenblum 2000, Gubitz et al. 1999). Similarly, it has been reported that the flash point of *Jatropha* oil and diesel is 2400 and 50°C respectively. In addition to this, cetane number of Bio-oil and Diesel is 51 and 50 respectively. Likewise, the Sulphur weight (%) of *Jatropha* oil and Diesel is 0.13 and 1.2 respectively (Radich 2004, Gubitz et al. 1999).

1.9 Objectives
The goal of my research work was to develop consortia of PGPR technology with appropriate doses of fertilizers to get maximum benefit in terms of fertilizer savings and better growth of *Jatropha*, increase soil fertility and nutrient status of the plant. For higher yield of *Jatropha* PGPR could also serve as a useful tool for alleviating salinity stress and this strategy could be applied for propogation of *Jatropha* in saline areas. The growth conditions, biomass production and seed yield of *Jatropha curcas* with the application of plant growth promoting rhizobacteria (PGPR), plant growth promoting consortia (PGPC), mycorrhizal fungi and other soil amendments like vermicompost and humic acid shall be studied.
Specific research tasks were to:

- Characterization of isolates according to their plant growth promoting potential
- Development of plant growth promoting consortium
- Study different soil amendments and microbial consortia for integrated nutrient management (INM) and growth promotion of *Jatropha curcas*
- Study effect of soil ameliorants and PGPR for integrated nutrient management (INM) and growth promotion of *Jatropha curcas* under salinity stress
- Identification of proteins responsible for salinity stress adaptation