1. Plant growth promoting rhizobacteria (PGPR)

Plant productivity is affected by innumerable biotic and abiotic factors. With an exponential increase in population, it is very essential to adopt novel methods and technologies to overcome the deleterious effects of both biotic and abiotic factors that affect crop productivity, so that it is feasible to feed the growing population. With increasing emphasis on green technologies that are environment friendly, researchers are looking for viable alternatives to conventional methods of crop production. Though a number of physical factors are directly or indirectly involved in plant growth and development, soil plays a major role in the process. The rhizosphere is a volume of soil around plant roots where plant growth is stimulated. It is a place where many biologically important functions and interactions take place. The rhizosphere is populated by a diverse range of microorganisms, and the bacteria colonizing this habitat are called rhizobacteria. Hiltner, (1904) discovered that the rhizosphere, i.e., the layer of soil influenced by the root, is much richer in bacteria than the surrounding bulk soil. Although the concentration of bacteria in the rhizosphere is 10 to 1000 times higher than that in bulk soil, it is still 100-fold lower than that on the average laboratory medium. Therefore, the lifestyle of rhizobacteria is best characterized as starvation. To exert their beneficial effects in the root environment, bacteria have to be rhizosphere competent, i.e., able to compete well with other rhizosphere microbes for nutrients secreted by the root and for sites that can be occupied on the root. These rhizospheric microbes benefit because plant roots secrete metabolites that can be utilized as nutrients. In the rhizosphere, a substantial amount of the carbon fixed by the plant, 5–21% (Marschner, 1995), is secreted, mainly as root exudate. Exudation can give both physical and chemical support to plants e.g. root mucilage reduce friction between root tips and soil and protect root from drying, strengthened the contact between the soil and root contribute to soil texture. Exudates from roots also attract microorganisms (Timmusk et al; 2005). These exudates are believed to determine which microorganisms colonize roots in the rhizosphere. It is now known that plant roots also generate electrical signals; it has been shown that zoospores of oomycetic pathogens take advantage of these signals for their movements towards the root surface.

The rhizobacteria may be present (i) in the soil surrounding roots, (ii) on the root surface or rhizoplane, (iii) in the root tissue, inhabiting spaces between cortical cells
and (iv) inside the cells in specialized root structures or nodules. There are in general active process and not passive chance encounters between soil bacteria and plant root. Root colonization is the process whereby bacteria multiply in the spermosphere in response to plant exuded nutrients and colonize in/on the developing root system in soil with a native microflora. Rhizobacteria may exert one of three effects on the inoculated host plant i.e. deleterious, natural, or beneficial. They induce changes in both root morphology and physiology, often enhancing the plant growth at various stages of development directly or indirectly. The bacteria associated with plant can act as deleterious rhizobacteria (DRB) or beneficial plant growth promoting rhizobacteria (PGPR). Hence PGPR is considered as functionally root colonizing beneficial bacteria (Yasar et al; 2011).

There are various means by which plant growth promoting bacteria can influence plant growth directly as by atmospheric nitrogen fixation, solubilization of minerals such as phosphorus, production of siderophores that dissolve and chelate the iron, or production of plant growth hormones that regulate plant growth at different stages of development. Plant growth–promoting rhizobacteria (PGPR) are universal symbionts of higher plants, which enhance the adaptive potential of their hosts through a number of mechanisms, such as the mobilization of recalcitrant soil nutrients, the control of phytopathogens and synthesis of phytohormones like indole acetic acid (IAA) (Mordukhova et al; 1991), gibberellic acid (Mahmoud et al;1984), cytokinins (Tien et al; 1979), ethylene (Glick et al; 1995), enzymes such as β-1, 3-glucanase (Fridlender et al, 1993), chitinases (Renwick et al; 1991) and antibiotics (Shanahan et al;1992). Indirect growth promotion occurs when PGPR promote plant growth by improving growth conditions. Indirectly they help in antibiosis, induced resistance to pathogen, iron scavenging and competition for nutrients and niche. It can happen directly by the production of the antagonistic substances, e.g. siderophores, hydrogen cyanide, proteases, antibiotics, and chitinases etc, or indirectly by inducing resistance to pathogens. Plant growth promoting rhizobacteria belonging to *Bacillus* and *Pseudomonas* genus have also been used in bio-antagonism (Gasoni et al; 1998).

### 1.1 Plant growth promotion by production of plant growth regulators

One of the well studied mechanisms by which PGPR promote plants growth is by synthesis of phytohormones. Molecular approaches using microbial and plant mutants
altered in their ability to synthesize or respond to specific phytohormones respectively have increased the understanding of the role of phytohormone synthesis as a direct mechanism of plant growth enhancement by PGPR (Glick, 1995).

A large number of PGPR strains have been found to produce indole acetic acid (IAA) (Patten and Glick, 1996). These auxins influence many cellular functions and are involved in initiation of lateral and adventitious roots, stimulation of cell division and in elongation of stems and roots (Yang et al; 2003). Root proliferation by bacteria-secreted IAA causes enhancement of nutrients uptake by the associated plants (Lifshitz et al; 1987). However, the observed responses of IAA on plant growth vary from one species of plant to another depending upon the indigenous hormonal level of the treated plants (Loper and Schroth, 1986, Mordukhova et al; 1991). Arshad and Frankberger, (1992) reported concentration dependent effect of IAA on plant growth, i.e. low concentration of exogenous IAA can promote, whereas high IAA concentrations can inhibit root growth.

Chemical structure of IAA

Another important phytohormone that controls plant growth and development is gibberellic acid. Gibberellins are tetra-cyclic diterpenoid acids that are involved in a number of developmental and physiological processes in plants (Crozier et al;2000, Davies,1995). These processes include seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (King and Evans, 2003, Sponsel, 2003).

Chemical structure of GA\textsubscript{1}, GA\textsubscript{3}, and GA\textsubscript{4}
Gibberellins are also implicated in promotion of root growth, root hair abundance, inhibition of floral bud, differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy and delay of senescence in many organs of a range of plant species (Bottini and Luna, 1993; Reinoso et al, 2002). In most (if not all) of these processes gibberellins act in combination with other phytohormones and additional regulatory factors, so that the signaling pathways are highly integrated (Trewavas, 2000). However, gibberellins are produced not only by higher plants and fungi (MacMillan, 2002) but also by bacteria (Atzorn et al; 1988; Gutierrez-Manero et al; 2001). In fungi and bacteria there is no known role for gibberellins, rather they seem to be secondary metabolites that may play a role as signaling factors towards the host plant (Yadav et al; 2011).

1.1.2 Plant growth promotion by enhancing nutrient availability

PGPR enhance plants growth directly by: fixation of atmospheric nitrogen that is transferred to the plants, production of siderophores that chelate iron and make it available to the plants root, solubilization of minerals such as phosphorus. Direct enhancement of mineral uptake due to increases in specific ion fluxes at the root surface in the presence of PGPR has also been reported (Bertrand et al; 2000).

Iron is essential for processes such as respiration. It accumulates in common mineral phases such as iron oxides and hydroxides (the minerals that are responsible for red and yellow soil colors) hence cannot be readily utilized by organisms (Kraemer Stephan et al; 2005). Microbes release siderophores to scavenge iron from these mineral phases by formation of soluble Fe$^{3+}$ complexes that can be taken up by active transport mechanisms. Siderophores are also important for some pathogenic bacteria for their acquisition of iron (Miethke and Marahiel, 2007), hence there are great evolutionary pressures put on pathogenic bacteria or fungi to obtain this metal. Siderophores are amongst the strongest binders to Fe$^{3+}$ known, with enterobactin being one of the strongest among these (Dertz and Kim, 2003).

Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate and rock phosphate (Goldstein, 1986). There are considerable populations of phosphate-solubilizing bacteria in soil and in plant rhizospheres (Speberg, 1958,
Alexander, 1977). A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizosphere in comparison with non rhizosphere soil (Raghu and MacRae, 1966) (Yasmin et al; 2011).

PGPR therefore help in plant growth promotion and help to overcome various biotic stresses such as disease (De Boer et al; 2003) as well as abiotic stresses such as drought and nutritional deficiency (Timmusk and Wagner, 1999). In the present study, paddy was selected as a model system to understand the influence of the rhizospheric bacteria on biotic and abiotic stress.

1.2 Paddy cultivation and its significance

Rice is one of the most important cereal crops in the world. Rice plants have been traced back to 5000 BC, but the practice of rice growing is believed to have originated in China, and southern-eastern Asia, in about 2000 BC. In Asia, more than two billion people get 60-70 per cent of their energy requirement from rice and its derived products. In India, rice is cultivated in an area of 44 million hectare with an average productivity of 2.0 tonnes per hectare. Demand for rice is growing every year and it is estimated that in 2025 AD the requirement would be 140 million tonnes. To sustain present food self-sufficiency and to meet future food requirements, India has to increase its rice productivity atleast by 3 percent per annum (Thiyagarajan and Selvaraju, 2001).

Rice is normally grown as an annual plant, although in tropical areas it can survive as a perennial and can produce a ratoon crop for up to 30 years (IRRI, 2008). The rice plant can grow to 1–1.8 m tall, occasionally more depending on the variety and soil fertility. It has long, slender leaves 50–100 cm long and 2–2.5 cm broad. The small wind-pollinated flowers are produced in a branched arching to pendulous inflorescence 30–50 cm long. The edible seed is a grain (caryopsis) 5–12 mm long and 2–3 mm thick. The nutritional value of rice is shown below in a tabular form.

<table>
<thead>
<tr>
<th>Rice (Nutritional value per 100 gm) (USDA-National nutrient database)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy</strong></td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Sugars</td>
</tr>
</tbody>
</table>
There are many varieties of rice; for many purposes the main distinction is between long- and medium-grain rice. The long-grain rice (high amyllose) tends to remain intact after cooking; medium-grain rice (high amyllopectin) becomes more sticky.

Data on worldwide production of paddy for three consecutive years is shown in the table below.


<table>
<thead>
<tr>
<th>Year</th>
<th>2007/08</th>
<th>2008/09</th>
<th>2009/10 Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Production (million metric ton)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bangladesh</td>
<td>28,800</td>
<td>31,000</td>
<td>30,000</td>
</tr>
<tr>
<td>Brazil</td>
<td>8,199</td>
<td>8,595</td>
<td>8,840</td>
</tr>
<tr>
<td>Burma</td>
<td>10,730</td>
<td>10,150</td>
<td>10,730</td>
</tr>
<tr>
<td>Cambodia</td>
<td>4,238</td>
<td>4,520</td>
<td>4,630</td>
</tr>
<tr>
<td>China</td>
<td>129,850</td>
<td>134,330</td>
<td>136,000</td>
</tr>
<tr>
<td></td>
<td>4,385</td>
<td>4,387</td>
<td>4,374</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Egypt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td>96,690</td>
<td>99,150</td>
<td>84,000</td>
</tr>
<tr>
<td>Indonesia</td>
<td>37,000</td>
<td>38,300</td>
<td>37,600</td>
</tr>
<tr>
<td>Japan</td>
<td>7,930</td>
<td>8,029</td>
<td>7,620</td>
</tr>
<tr>
<td>South</td>
<td>4,408</td>
<td>4,843</td>
<td>4,500</td>
</tr>
<tr>
<td>Nigeria</td>
<td>3,000</td>
<td>3,200</td>
<td>3,400</td>
</tr>
<tr>
<td>Pakistan</td>
<td>5,700</td>
<td>6,300</td>
<td>6,000</td>
</tr>
<tr>
<td>Philippines</td>
<td>10,479</td>
<td>10,753</td>
<td>10,710</td>
</tr>
<tr>
<td>Thailand</td>
<td>19,300</td>
<td>19,400</td>
<td>20,000</td>
</tr>
<tr>
<td>Vietnam</td>
<td>24,375</td>
<td>24,430</td>
<td>23,795</td>
</tr>
<tr>
<td>Others</td>
<td>31,970</td>
<td>31,765</td>
<td>34,400</td>
</tr>
<tr>
<td>Subtotal</td>
<td>427,054</td>
<td>439,152</td>
<td>426,599</td>
</tr>
<tr>
<td>States</td>
<td>6,344</td>
<td>6,515</td>
<td>7,056</td>
</tr>
<tr>
<td>World Total</td>
<td>433,398</td>
<td>445,667</td>
<td>433,655</td>
</tr>
</tbody>
</table>

It is evident from the table shown above that more than 90 percent of the world's rice is grown and consumed in Asia, where people typically eat rice two or three times daily. To plow 1 hectare of land in the traditional way, a farmer and his buffalo must walk 80 km. It takes 5,000 liters of water to produce 1 kg of irrigated rice. More than 140,000 varieties of cultivated rice (*Oryza sativa*) are thought to exist but the exact number remains a mystery. Three of the world's four most popular nations are rice-based societies: People's Republic of China, India, and Indonesia. Together, they have nearly 2.5 billion people almost half of the world's population. The average Asian consumer eats 150 kg of rice annually compared to the average European who eats 5 kg. Every year, 50 million people are added to Asia's soaring population of 3.5 billion. Improved varieties are planted on three fourths of Asia's rice land and are responsible for producing most of the continent's rice. Asia is home to 250 million rice farms. 65 kilos of rice are milled annually for every person on earth. Production of such an
important cereal crop is affected by a number of biotic, abiotic and socio-economic factors.

1.2.1 Factors affecting paddy production

Paddy is one of the world's most important cereal crops, and its protection from disease is vital to the many millions people dependent on it as their staple food. The main constraints to rice production are a combination of abiotic, biotic, and socioeconomic factors. Abiotic factors include drought, flood, cold temperature and poor soil fertility, while biotic factors are insects, diseases, and weeds. Socioeconomic factors include labor shortages and lack of access to credit and markets. Annual drought and flooding are the most serious constraints to rice cultivation. Late season drought alone can reduce grain yields by 30%. In the upland environment, drought at seeding is an important production constraint. Frequently occurring floods result in further loss of productive capacity through soil erosion (Fukai et al; 1998).

Biotic constraints (insects, diseases, and weeds) are ranked to be the most serious constraints affecting rice production in the uplands and the third most serious among lowland farmers. Brown plant-hopper, stem-borer, rice bug, golden apple snail, gall midge, and white grub (particularly in the drought period) are some of the insect pests reported to result in significant crop losses during both the wet and dry seasons. Blast, bacterial leaf blight, brown spot, bakanae in the lowlands, and nematodes in the uplands also reduce yields substantially. In the upland ecosystem, weeds and rodents are the two major constraints that result in significant economic losses. It is estimated that at least 15% of the annual harvest is lost due to rodents.

Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. The non-living variable must influence the environment beyond its normal range of variation to adversely affect the population performance or individual physiology of the organism in a significant way. Abiotic stress is essentially unavoidable and may affect animals, but plants are very sensitive to environmental fluctuations and are especially dependent on environmental factors. Abiotic stress is the most harmful factor that affects the growth and productivity of crops worldwide (Gao et al; 2007). It has been claimed that abiotic stress causes the most crop loss than any other factor and that most major crops such as rice are reduced in their yield by more than 50% from their potential yield (Wang et al; 2007).
1.3 Role of PGPR in alleviation of biotic stress

Plant growth and yield are influenced by a myriad of abiotic and biotic factors. Among the biotic stresses, pathogenic microorganisms are a major threat to plant health and yield. As agricultural production intensified over past few decades, farmers became more and more dependent on agrochemicals as a relatively reliable method for crop protection. However, increasing use of chemicals leads to indiscriminate resistance, environmental pollution, impact on several other non-targeted organisms, high level of chemicals in food chain and harmful effect on economically valuable vegetation (De Weger et al; 1995). Biological control is therefore being considered as an alternative or supplemental way of reducing the use of chemicals in agriculture. The potential use of plant associated bacteria as agents stimulating plants growth, managing soil and plant health is well documented (Sturz et al; 2000). The widest groups of such bacteria are PGPR (Klopper et al; 1997), which colonize the root surfaces and closely adhering soil interface of the rhizosphere. These rhizobacteria have immense potential in agriculture for use as biofertilizer, biocontrol agent and in bioremediation due to their plant growth-promoting ability, antagonistic activity and degradation of pollutants (Ahmad et al; 2008).

1.3.1 Mechanisms of biocontrol by PGPR

The biological control of plant diseases is emerging as a popular and effective alternative to conventional chemical control measures. In the past, extensive work has been done by different workers on biological control of plant diseases using microbes. Research with free living bacteria as agricultural inoculants has the same historical genesis as Azospirillum research. By the late 1800s beneficial effects of the symbiotic rhizobia were well established on legumes and researchers began to ask the question, “Can the same type of plant benefits be realized on non legumes with other soil bacteria”? Pioneering work on non rhizobial system was done in Russia with bacterization of seeds i.e. treatment with culture of bacteria to improve plant growth, which was proposed as early as 1895 using Bacillus spp. and in 1909 by using Azotobacter chroococcum (David, 2007). Multiple studies with these inoculants were reported during the 1960s in journals from the Soviet Union and Eastern Europe which were reviewed by Brown (1974). These studies supported the conclusion that bacterization generally resulted in yield increase up to 10% for cereal crops and 15%
to 50% with various vegetables. In the mid 1960 and early 1970s bacterization work in India was reported by using the inoculants from Soviet Union and indigenous strains of *Pseudomonas* and *Beijerinkia* (Balasundaram and Sen 1971). Even when the bacterization strain is selected from among the indigenous rhizosphere microflora, such as with *Pseudomonas*, the inoculated strain will encounter intense competition from indigenous bacteria immediately upon entry to non pasteurized field. This logic leads to the theory of “Microbial equilibrium”, that states that rhizosphere microflora is in a state of dynamic equilibrium such that the population of any introduced microorganism will decline rapidly following inoculation into soil. Intense research to monitor specific bacteria in the past few years has led to several new marking systems which can measure root colonization by bacteria (Shah, 2006). When developing a concept of root colonization it is critical to note that the capacity to colonize roots is strain specific (Zhao et al; 2011).

**Antibiotic production**

The most widely studied group of rhizobacteria with respect to the production of antibiotics is that of the fluorescent *pseudomonas*. There are several biocontrol systems in which one or more antibiotics have been used to play a role in disease management. Production of antibiotics is closely related to the overall metabolic system of the organism, which in turn is dictated by nutrient availability and other environmental stimuli, such as type of carbon source and supply, major and minor minerals, pH, temperature, and other parameters (Ownley et al; 2003). The varied arsenal of bio-antagonistic strains may enable the pathogen to perform their ultimate objective of disease suppression under different environmental conditions.

**Competition for nutrients**

Microorganisms compete with each other for nutrient availability. Among several mechanisms by which plant growth promoting rhizobacteria can inhibit pathogens, one of them is competition for nutrition. Competition for nutrients exerted by root exudates is probably a significant factor in many interactions between plant growth promoting rhizobacteria and other pathogens. Populations of bacteria established on a plant root could act as a sink for nutrients in the rhizosphere, hence reducing the nutritional availability for pathogen stimulation or subsequent colonization of the root. This phenomenon is used by fluorescent *pseudomonas* because they have rapid growth rate in soil environment due to their nutritional versatility (Bais et al; 2006).
Parasitism and production of extracellular enzymes

One of the important biocontrol mechanisms for plant disease control is parasitism and production of extracellular enzymes. The ability of bacteria, especially *actinomycetes*, to parasitize and degrade spores of fungal plant pathogens is well established (Nelson et al; 1995). Considerable effort has been put in to identify cell wall degrading enzymes produced by biocontrol strains of bacteria even though relatively little direct evidence for their presence and activity in the rhizosphere has been obtained. Chitinolytic enzymes produced by bacteria such as *Bacillus cereus* (Chang et al; 2003) and *Pantoea agglomerans* are involved in biocontrol of fungal pathogens (Bonaterra et al; 2003).

1.3.2 PGPR as a biocontrol agent against rice blast.

In the pre-war period, diseases of rice were practically unimportant in Tropical Asia where ancient varieties were traditionally grown in soils of relatively low fertility. However, with the increase in demand for world rice supplies, there has been an awakening interest to maximize production using improved varieties, high fertilization and other intensive cultural practices. High cultural regimes, therefore, have led to a great increase in the occurrence and severity of diseases affecting rice in several countries, especially in Tropical Asia.

Rice suffers from a number of fungal and other diseases in India. Of the common fungal diseases, blast caused by *Magnaporthe grisea* is the most important disease. In some regions of the country, this disease is endemic causing severe damage to the crop every year. These areas are confined to higher elevations of 2000 – 5000 ft or in the valleys where conditions are favorable for blast development throughout the crop season. In other parts of India, blast is seasonal in occurrence, severity of which depends upon prevailing weather factors.

Causes and development of rice blast

The study of relation of metrological factors on development of rice diseases has been in progress at the Central Rice Research Institute in respect of blast. As regards blast it was found that a coincidence of low temperature of 20 – 26 °C and below, especially 24°C and below with high relative humidity of 90 % and above during the susceptible stages of crop growth (seedling, tillering and neck emergence) was accompanied by blast outbreak.
Blast is generally considered as most important world-wide disease of rice due to its widespread distribution and the potential to cause up to 50% yield loss when conditions are favorable for its occurrence. Blast can infect rice from the seedling stage through maturity. Infection results in lesions on most of the plant parts including leaves, leaf collar, stems, nodes, panicles and grain. The leaf sheath is usually not infected. The disease may also be called leaf blast. Rice blast disease is distributed in about 85 countries in all continents where the rice plant is cultivated, in both lowland and upland conditions. Although the damage is very much influenced by environmental factors, this disease is recognized as one of the most serious diseases of the rice plant worldwide.

**Life cycle of *Magnaporthe grisea***

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*Magnaporthe grisea* spores

Leaf sheath of paddy showing symptoms of blast disease
Infection Mode

*Magnaporthe grisea* infects rice plants in a manner typical of other foliar pathogens. Asexual spores, called conidia, are dispersed in moist air and attach tightly to the leaf surface (Hamer et al; 1988). In a drop of water, a conidium produces a germ tube that grows and differentiates in a specialized infection structure called an appressorium that adheres tightly to the plant surface (Bourett and Howard, 1990). This specialized cell generates enormous turgor pressure that is used to penetrate the underlying plant surface (Howard et al; 1991). Once inside the plant, bulbous, lobed infection hyphae grow in and between plant cells. Eventually a lesion develops that under conditions of high humidity will yield mycelia that sporulate and release more conidia to reinitiate the infection cycle. Fungal pathogens like *M. grisea* commonly rely on thigmotrophic sensing mechanisms to decipher surfaces appropriate for appressorium formation (Hoch and Staples, 1991). The rice blast fungus senses the presence of hydrophobic surfaces that mimic the rice leaf surface (Hamer et al; 1988, Bourett and Howard, 1990).

1.3.3 PGPR mediated induced systemic resistance (ISR)

Rice is subject to several destructive diseases for the control of which dependence on techniques like, use of resistant variety, crop rotation, and host resistance alone has been found to be unreliable and disappointing. It thus seems that chemical control offers greater promise and will constitute an important weapon in reducing crop losses caused by rice diseases. But there are several drawbacks in prolonged use of chemical methods as it damages the natural beneficial insects, environment and also contaminate the natural resources.

In the context of increasing international concern for food and environmental quality, the use of PGPR for reducing chemical inputs in agriculture is potentially important. PGPRs have been applied to crops in various forms to enhance growth, seed emergence and crop yield, and a few have been commercialized (Herman et al; 2008, Minorsky, 2008). Some root colonizing non-pathogenic rhizobacteria may also trigger disease resistance in the host plant, a phenomenon that has been termed induced systemic resistance (ISR) (Van Loon et al; 1998). ISR is a plant mediated mechanism that starts in the roots and extends up to the shoots. It is effective against different types of plant pathogens. ISR induced by PGPR has not yet been reported for
biological control of diseases but has attracted interest because it has led to disease reduction and promotion of plant growth and yield (Chanway et al; 2000).

### 1.3.4 Mechanism of ISR induction by PGPR

ISR triggered in the plant by rhizobacteria is referred to as rhizobacterial medicated ISR (Van Loon et al; 1998). It is brought about by PGPR through fortification of physical and mechanical strength of the cell wall as well as changing the physiological and biochemical reaction of host leading to the synthesis of defense chemicals against the pathogen. PGPR could act as strong elicitors of plant defense reaction (M’Piga et al; 1997).

The utilization of a plant’s own defense mechanism is the subject of current interest in the management of pests and diseases. Induction of plant defense genes by prior application of inducing agents is called induced resistance (Hammerschmidt and Kuc, 1995). The defense gene products include peroxidase (PO), polyphenol oxidase (PPO) that catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) that is involved in phytoalexin and phenolics biosynthesis. Other defense enzymes include PR proteins such as β-1, 3-glucanases (PR-2 family) and chitinases (PR-3 family) which degrade the fungal cell wall and cause lysis of fungal cells. Chitin and glucan oligomers released during degradation of fungal cell wall act as elicitors that elicit various defense mechanisms in the plants (Frindlender et al; 1993). Induction of defense enzymes makes the plant resistant to pathogen invasion (Van Loon et al; 1998). Excellent inducers include pathogens (Dalisay and Kuc, 1995) and non-pathogenic plant growth-promoting rhizobacteria (PGPR) (Ramamoorthy et al; 2002). Phenylalanine ammonia lyase (PAL) catalyzes the deamination of L-phenylalanine to yield trans-cinnamic acid, the common precursor for biosynthesis of phenolic derivatives like flavonoids, monolignols, and salicylates that are essential for adaptive, vascular, and reproductive plant development (Hahlbrock and Grisebach, 1979). Phenylalanine is the starting compound used in the flavonoid biosynthesis. Lignin is derived from phenylalanine and from tyrosine (Daayf et al; 1997). De Meyer et al. (1999) reported that rhizosphere colonization by *P. aeruginosa* 7 NSK2 activated PAL in bean roots and increased the salicylic acid levels in leaves. Induction of PAL by fluorescent *pseudomonas* was reported in cucumber against *P. aphanidermatum* (Chen et al; 2000) and tomato against *F. oxysporum* f. sp. *lycopersici* (Ramamoorthy et al; 2002). Accumulation of PAL was higher in the
combination of *Pseudomonas* strains (EPB22-Pf1) treated banana (Harish, 2005) and mung bean plants (Thilagavathi et al; 2007). 

β-1, 3-glucanases are involved in pathogenesis in plants. They are classified as a family 17 hydrolase based on structural criteria (Henrissat and Davies, 1997), and preferentially hydrolyze 1,3-β-D-glycosidic linkages in (1 - 3)-β-D- and (1-3),(1-6)-β-D glucans in the cell walls of many pathogenic fungi. Apart from their role in plant defense, β-1,3-glucanases are involved in diverse physiological and developmental processes such as endosperm formation (Wu and Bradford, 2003), somatic embryogenesis in Cichorium (Helleboid et al; 2000), microsporogenesis (Worrall et al; 1992), pollen development (Hird et al; 1993), seed germination (Leubner-Metzger, 2005), flower formation (Akiyama et al; 2004), and the response to wounding and abiotic stress (Obregon et al; 2001). The subcellular localization of hydrolytic enzymes in plants can provide important information about the role of hydrolytic enzymes during defense against pathogens (Wubben et al; 1992). Various studies have revealed the higher induction of chitinase and β-1,3-glucanase in tea plants treated with *P. fluorescens* Pf1 against blister blight disease. These findings are in agreement with the induction of β-1,3-glucanase activity against *Peronospora tabacina* on tobacco (Ye et al; 1990).

Chitinases are enzymes that hydrolyze the N-acetylglucosamine polymer of chitin, and they occur in diverse plant tissues over a broad range of crop and non crop species. The enzymes may be expressed constitutively at low levels but are dramatically enhanced by numerous abiotic agents (ethylene, salicylic acid, salt solutions, ozone, UV light) and by biotic factors (fungi, bacteria, viruses, viroids, fungal cell wall components, and oligosaccharides). Different classes of plant chitinases are distinguishable by molecular, biochemical, and physicochemical criteria. In vivo, rapid accumulation and high levels of chitinases (together with numerous other pathogenesis-related proteins) occur in resistant tissues expressing a hypersensitive reaction, though high levels also can occur in susceptible tissues. The expression of chitinase in combination with one or several different antifungal proteins should have a greater effect on reducing disease development, given the complexities of fungal-plant cell interactions and resistance responses in plants. Maurhofer et al; (1994) reported that PGPRs could reduce pathogen viability and
induction of systemic resistance by *P. fluorescens* was correlated with accumulation of chitinase.

Polyphenol oxidases (PPO) are a group of copper proteins that are widely distributed from bacteria to mammals (Robb, 1984). They catalyze the oxidation of hydroxyphenols to their quinone derivatives, which then spontaneously polymerize. The 3 types of proteins related to PPOs are catechol oxidase, laccase, and cresolase (Yelena et al; 1996). They catalyze 2 reactions: the hydroxylation of monophenols to o-diphenols (monophenolase activity) and the oxidation of o-diphenols to o-quinones (diphenolase activity). Polyphenol oxidase oxidizes phenolics to highly toxic quinones and is speculated to be involved in the terminal oxidation in the diseased plant tissue which was attributed for its role in disease resistance. Ganeshamoorthi et al; (2008) reported that induction of PPO was higher in plants treated with a bioformulation mixture than individual bioformulation treatments. Radjacommare, (2000) reported that *P. fluorescens* induced PPO activity in rice against *R. solani*. Chen et al; (2000) reported that various rhizobacteria and *P. aphanidermatum* induced PPO activity in cucumber root tissues. Peroxidases (PO) are heme-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyze a number of oxidative reactions. Most heme- peroxidases follow the reaction scheme:

\[ \text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow [\text{Fe}^{4+}=\text{O}] \text{R}' (\text{Compound I}) + \text{H}_2\text{O} \]

\[ [\text{Fe}^{4+}=\text{O}]\text{R}' + \text{substrate} \rightarrow [\text{Fe}^{4+}=\text{O}]\text{R} (\text{Compound II}) + \text{oxidised substrate} \]

\[ [\text{Fe}^{4+}=\text{O}]\text{R} + \text{substrate} \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \text{oxidised substrate} \]

In this, the enzyme reacts with one equivalent of \( \text{H}_2\text{O}_2 \) to give (\( \text{Fe}^{4+}=\text{O} \) \( \text{R}' \) (compound I). This is a two-electron oxidation/reduction reaction where \( \text{H}_2\text{O}_2 \) is reduced to water and the enzyme is oxidized (Schuller et al; 1996). Peroxidases are found in bacteria, fungi, plants and animals and can be viewed as members of a superfamily consisting of 3 major classes (Welinder KG, 1992) Class III comprises the secretory plant peroxidases, which have multiple tissue-specific functions as removal of hydrogen peroxide from chloroplasts and cytosol; oxidation of toxic compounds; biosynthesis of the cell wall; defense responses towards wounding; indole-3-acetic acid (IAA) catabolism; ethylene biosynthesis; and so on. Plant peroxidases are monomeric glycoproteins containing 4 conserved disulphide bridges and 2 calcium ions. Akimova
et al; (2002) demonstrated that pea responded to *Rhizobium* inoculation by enhancing peroxidase activity.

### 1.4 Role of PGPRs in alleviation of abiotic stress

Abiotic components are the nonliving components of the biosphere, consisting of chemical and geological factors, such as rocks, minerals, soil and physical factors such as weather. Soil is a major component of the abiotic factors. These factors include soil texture, soil air, soil temperature, soil water, soil solution and pH, together with soil organisms and decaying matter. A traditional view of the influence of soil is that it provides an opportunity, or a constraint on the type of cropping system that can be implemented and its productivity. Another view is that 'the soil' combines various properties which interrelate and are directly influenced by the procedures of cropping. Each of these properties may affect others and that all can directly and indirectly reduces plant performance, as well as affect other aspects including erosion, salinization and acidification. Among them, soil salinization is a major worldwide problem. In India alone, thousands of square kilometers have been severely salinized. The major factor in the development of saline soils is lack of precipitation. Most naturally saline soils are found in (semi) arid regions and climates. In India, especially in Gujarat, a considerably large area is affected by salinity as indicated in the map. It is interesting to note that not only the coastal regions, but inlands also are affected by salinity. This is due to the extensive irrigation from the rivers.

**Map showing areas affected by soil salinity and soil erosion in the state Gujarat**
Saline soils are soils that have a high salt content. The predominant salt is normally sodium chloride (NaCl, "table salt"). Saline soils are therefore also sodic soils but there may be sodic soils that are not saline, but alkaline. Saline soils are a common feature and an environmental problem in irrigated lands in arid and semi-arid regions. They have poor or little crop production. The problems are often associated with high water tables, caused by a lack of natural subsurface drainage to the underground. Poor subsurface drainage may be caused by insufficient transport capacity of the aquifer or because water cannot exit the aquifer for instance, if it is situated in a topographical depression. Soil salinity affects vast areas of farming land and the area of salt affected land is increasing rapidly. The cost in lost agricultural production is estimated at Rs 1600 crore per year due to salinity. Plants being sessile, their growth and yield are strongly influenced by abiotic stress which presents a major challenge for sustainable food production as it reduces the potential yields as high as by 70% in crop plants. All plants do not respond to salinity in a similar manner; some crops can produce acceptable yields at much greater soil salinity than others. This is because some are better able to make the needed osmotic adjustments enabling them to extract more water from a saline soil. The ability of the crop to adjust to salinity is extremely useful. A strategy to acquire much water is essential for plant growth under water deficit conditions. To overcome water deficit, plants have developed mechanisms of physiological adaptation, such as improvement of water use efficiency by regulation of stomatal closure, development of root system to acquire water, accumulation of osmoprotectants and control of water permeability by aquaporins (Jang et al; 2004). Salinity stress also decreases photosynthetic capacity due to the osmotic stress and partial closure of stomata. Plants can suffer from membrane destabilization and general nutrient imbalance. But in the last decade there were number of reports on the beneficial effects of microorganisms such as Pseudomonas, Bacillus, Pantoea, Burkholderia, Rhizobium etc. in enhancing the tolerance of crops such as sunflower, maize, wheat, chickpea, groundnut, spices and grapes to drought, salinity, heat stress and chilling injury under controlled conditions (Arshad et al; 2008).

1.4.1 Role of osmoprotectants in saline stress
Salt stress results in a wide variety of physiological and biochemical changes in plant. Among them, accumulation of low molecular weight solutes, such as proline and...
betaine derivatives is one (Yancy et al; 1982). Plants accumulate proline and betaine derivatives to mitigate detrimental effects of salt stress, by lowering water potential. The accumulation of compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, and thus buffering the immediate effect of water shortage. High salt concentration causes an imbalance of cellular ions resulting in ion toxicity, osmotic stress and production of active oxygen species (Cheesman, 1988).

More specifically soil–borne Pseudomonas species have received special attention because of their catabolic versatility, root colonizing ability and capacity to produce a wide range of enzymes and metabolites that help the plants withstand varied biotic and abiotic stress conditions (Vessey, 2003). While a number of studies have elucidated the role of PGPRs in plant growth under unfavorable conditions. Kohler et al; (2008) reported that plant inoculated with PGPR and AM fungus, alone or in combination, can confer salinity tolerance due to changes in the activity of antioxidant enzymes (catalase and total peroxidase activities) and the accumulation of solutes (proline, glycine betaine and soluble sugars) (Zhao et al; 2011).

Among the many quaternary ammonium compounds known in plants, glycine betaine (GB) occurs most abundantly in response to dehydration stress (Yang et al; 2003). GB is abundant mainly in chloroplast where it plays a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Genard et al; 1991). In higher plants, GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde (Rhodes and Hanson, 1993). Choline is converted to betaine aldehyde, by choline monooxygenase (CMO), which is then converted to GB by betaine aldehyde dehydrogenase (BADH). The reaction is shown below:
Of the many quaternary ammonium compounds (QACs) which function as effective compatible osmolytes in plants subjected to salt or drought stress, glycine betaine occurs widely in plants (Venkatesan and Chellappan, 1998). It was reported that betaine arises as a result of de-novo synthesis from one and two carbon precursors during water stress (Hanson and Nelson, 1978). Several soil bacteria produce osmolytes to protect themselves against the frequent fluctuation in osmotic conditions. A close relative to *Paenibacillus polymyxa*, *Bacillus subtilis*, produces glycine betaine (Lucht and Bremer, 1994).

Proline is one of the well-known osmoprotectant and its accumulation is widely observed in various organisms under salt stress. Proline accumulation is regulated by multiple factors, such as its synthesis, catabolism, utilization for protein synthesis and transport from other tissues. Expression of D1-pyrroline-5-carboxylate synthetase (P5CS) and proline dehydrogenase (PDH), the key genes in proline synthesis and catabolism, respectively, is regulated in a coordinate manner in response to environmental stress (Yoshiba et al; 1997). Improved accumulation of free proline was shown to confer salt and drought tolerance in tobacco and this result supported that free proline functions to regulate cellular osmotic balance (Kishor et al; 1995). Additionally, proline is also utilized for protein synthesis, and large part of hydroxyproline, a derivative of proline through hydroxylation, is found in structural proteins, such as collagen in animals or hydroxyproline rich protein in plants (Hall et al; 2002, Myllyharju et al; 2003). Proline and hydroxyproline in structural proteins are clearly distinguished from free proline, which serves to regulate osmotic adjustment. Increased accumulation of free proline was enhanced in the root apical region of maize seedlings in response to water stress (Verslues et al; 1999, Raymond et al; 2002).

1.4.2 Role of PGPRs on antioxidant level

Plants functioning in an aerobic environment are often subjected to continuous threat from molecular oxygen which is due to toxic reactive oxygen species (ROS) like superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH). These reactive oxygen species have the capacity to degrade almost all cell components including membrane lipids, proteins and DNA (Casano et al; 1997). To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide
dismutase (SOD), peroxidase (POX) and catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), Acid phosphatase (AP), and non-enzymatic antioxidants such as ascorbate, glutathione and $\alpha$-tocopherol (Del Rio et al; 2003). However, levels of antioxidant enzyme and antioxidant concentrations are frequently used as indicators of oxidative stress in plants (Mittler, 2002). Induction of antioxidant enzyme was involved in the ability of the PGPR strain to increase the tolerance of lettuce grown under severe salt stress. Therefore, inoculation with selected PGPR could serve as a useful tool for alleviating salinity stress in salt-sensitive plants (Kohler et al; 2009).

Reactive $O_2$ species (ROS) are produced in both unstressed and stressed cells. Plants have well-developed defense systems against ROS, involving both limiting the formation of ROS as well as instituting its removal. Under unstressed conditions, the formation and removal of $O_2$ are in balance. However, the defense system, when presented with increased ROS formation under stress conditions, can be overwhelmed. Within a cell, the superoxide dismutase (SOD) constitutes the first line of defense against ROS. $O_2^-$ is produced at any location where an electron transport chain is present, and hence $O_2$ activation may occur in different compartments of the cell (Elstner, 1982), including mitochondria, chloroplasts, microsomes, glyoxysomes, peroxisomes, apoplasts, and the cytosol. The increased level of antioxidant enzymes SOD and PO has been detected in rice under salt stress condition (Lee et al; 2001)

Catalase (CAT) a tetrameric heme-containing enzyme, removes toxic hydrogen peroxide generated by a variety of metabolic reactions and environmental stresses in peroxisomes (Willekens et al; 1995). In higher plants, catalase is present in all differentiated peroxisomes including glyoxysomes, leaf peroxisomes, cotyledonary peroxisomes, root peroxisomes and unspecialized peroxisomes (Kamada et al; 2003). Catalase, which degrades $H_2O_2$ into water and oxygen, is one of the major antioxidant enzymes (Scandalios et al; 1997). Accumulation of catalase evidence that catalase plays an important role in plant defense, aging, and senescence. Catalase, are the key antioxidant enzyme, which exert their effect through different pathways explained by different workers. Nautiyal et al; (2008) in his study find an increased catalase activity in all the selected vegetables, irrespective of the site of inoculation of PGPR (soil inoculation), which indicates that catalase is induced by PGPR.
Peroxidase (PO) is involved in the production or modulation of active oxygen species which may play various roles directly or indirectly in reducing pathogen viability and spread as well as enhanced ROS level in plant (Lamb and Dixon, 1997). Saravanakumar et al; (2007) observed that, foliar sprays of *Pseudomonas fluorescens* induced higher activities of PO and PPO in tea plant against the blister blight pathogen compared to the untreated control. Ramamoorthy et al; (2002), reported enhanced resistance of tomato and hot pepper to Pythium diseases by seed treatment with *Pseudomonas fluorescens* by enhanced PO activity, compared to the non-bacterized pathogen control plants.

Glutathione reductase (GR) is thought to be a bottleneck in the antioxidative cascade of plants, since it is present in lesser amounts compared to other enzymes of the defense system against free radical attack (Polle, 2001). The thiol-containing tripeptide glutathione is widespread in plant cells in high concentrations (Alsch er, 1989). Glutathione functions as an antioxidant and has a role in the detoxification of xenobiotics and air pollutants and the removal of toxic free-radical and hydroperoxides in the ascorbate-glutathione cycle (Alsch er, 1989). Glutathione reductase is ubiquitous in living organisms (Smith et al; 1989). This enzyme is coupled directly with glutathione peroxidase in animals and indirectly with ascorbate peroxidase via dehydroascorbate reductase in higher plants. It seems likely that glutathione reductase participates in the maintenance of a large pool of glutathione in the reduced form and the acceleration of the H$_2$O$_2$, scavenging pathway in vivo (Smith et at; 1989). Similar reports on the effect of metals on the GR activity of legumes (Reddy et al; 2005) and other detoxifying agents produced by rhizobia are reported by Figueira et al; (2005).

The level of lipid peroxidation, measured as malondialdehyde (MDA) content, has been considered as an indicator of salt-induced oxidation in cell membranes and a tool for determining salt tolerance in plants (Hernández and Almansa, 2002). Lipid peroxidation rate was found to increase with increase of salt stress in sensitive maize cultivars (Azevedo Neto et al; 2006, Arora et al; 2008). Drought and salt stress leads to an increase of reactive oxygen species (ROS) which include singlet oxygen, superoxide anion, hydroxyl radical and hydrogen peroxide. High concentration of ROS inhibits the repair ability of photosynthesis system damage (Lawlor, 2002). The extent of damage to the membrane is monitored by undergoing peroxidation of
polyunsaturated fatty acids in the membrane. The enhance free radical formation and lipid peroxidation under oxidative stress and may have also brought about an increase in membrane permeability or loss of membrane integrity in plant cells (Guo et al; 2006). Nautiyal et al; (2008) reported that plant extracts inoculated with PGPR had better lipid peroxidation inhibition, compared with non-inoculated plant extracts. Acid phosphatase (AP) enzymes are highly expressed in plants and especially in plant tissues such as seeds, bulbs, roots, tubers, coleoptiles and leaves. Leaf phosphatases from beans (Phaseolus vulgaris) (Tejera Garcia et al; 2004), soybean (Glycine max) (Staswick et al; 1994) and rice (Oryza sativa) (Shih and Kao, 1997) have been characterized. In some plants the increased acid phosphatase activity in leaves is associated with phosphorus deficiency symptoms (Duff et al; 1994).

1.5 Induction of the gene expression under biotic and abiotic stresses in plants by PGPR

In agricultural systems, the biotic and abiotic stresses such as pathogen infection, drought, low temperature and salinity in particular are responsible for most of the reduction that differentiates yield potential from harvestable yield (Boyer, 1982). Plants draw on a large repertoire of defense responses when infected by pathogens. The best studied of these responses is the synthesis of new proteins that can have direct or indirect action on the course of pathogenesis. These proteins include enzymes involved in phenylpropanoid and flavonoid metabolism (Lamb et al; 1989), peroxidases (Lagrimini et al; 1987) β-1,3 glucanases and chitinases (Abeles et al; 1971), hydroxyproline-rich glycoproteins (Showalter et al; 1989), and a diverse group of acidic, extracellular proteins known collectively as PR' proteins (Carr et al; 1985). Among the proteins having known enzymatic function, the β-1, 3-glucanases are particularly interesting because they are hormonally and developmentally regulated in uninfected plants (Felix et al; 1993) and are thought to protect plants from fungal infection (Boller et al; 1988). Abiotic stress as salt stress, can lead to changes in development, growth, productivity and severe stress may threaten survival. Abiotic stresses have been shown to cause accumulation of many intracellular substances, including nucleic acids, proteins, carbohydrates and amino acids. After the introduction of molecular biological techniques into plant biology, a great deal of effort went into the identification of stress-inducible genes, such as RD29A, using
differential screening or differential display techniques for various plant species, including *Arabidopsis*. These studies succeeded in isolating genes that are presumed to function in stress responses and tolerance. Over-expression of some of these genes in plant confers some abiotic stress tolerance (Bartels and Sunkar, 2005; Umezawa et al; 2006). More importantly, using the expression of such inducible genes as markers, an overall scheme of transcriptional regulation was developed. In the emerging picture, transcriptional activation occurs at distinct time points in response to stress stimuli. The various induction phases for stress-inducible genes are due to their varying dependency on de novo synthesis of proteins or signaling molecules, such as abscisic acid (ABA) (Yamaguchi- Shinozaki and Shinozaki, 2006). These findings suggest that abiotic stress responses are never simple, and that each induction phase may be controlled by a different signaling mechanism and different transcription factors. Studies with transgenic plants support the notion that altered gene expression can lead to improvements in tolerance (Apse et al; 1999, Zhao et al; 2011).

In an earlier study on induced systemic tolerance (IST) to drought, Timmusk et al; (1999) reported that inoculation with the PGPR *Paenibacillus polymyxa* enhanced the drought tolerance of *Arabidopsis thaliana*. RNA differential display on parallel RNA preparations from *Paenibacillus polymyxa*-treated and untreated plants revealed the mRNA transcriptions of a drought-response gene, EARLY RESPONSIVE TO DEHYDRATION 15 (ERD15). Similarly, Rocha et al; (2007) reported the differential expression of as many as 93 sugarcane genes, including well-known drought-responsive genes such as *MRB* and *WRKY* transcription factors by drought treatment. However, treatment of the same plant with beneficial endophytic bacteria (*Herbaspirillum* spp. and *Gluconacetobacter diazotrophicus*) resulted in the induction of resistance (R) and salicylic acid biosynthesis genes. In contrast to endophytic bacteria inoculation, however, expression of the gene was unaffected by salt stress. This suggests a variance in plant gene expression patterns under biotic or abiotic stress conditions, relative to their co-regulation as previously observed by Timmusk and Wagner (1999). PGPR products have not been associated with major changes in defense gene expression (Van Loon, 1998) though some strains of *Bacillus* and *Chryseobacterium* have been found to activate defense-related marker genes (Ramos et al; 2008). Herman et al; (2008) reported that the PGPR product primed plants to respond more quickly and to a greater degree after inoculation, though the effect was
inconsistent. Alfano et al; (2007) found several tomato genes up-regulated via *Trichoderma* induction of systemic resistance, including extensin and osmotin. Possibly these markers could provide more information regarding the PGPR–tomato interaction as *Trichoderma* spp. have been found to induce defenses in a manner similar to PGPR (Conrath et al; 2006). Verhagen and associates (2004) have reported the transcriptome analysis of *Arabidopsis* plants colonized with the well-studied *Pseudomonas fluorescens* strain WCS417r. In the analysis, 97 genes were identified as WCS417r-responsive genes in roots.

It is evident from the foregoing account that the use of PGPR is a safe and economical option for the management of biotic and abiotic stresses in crop plants. The following are the specific objectives of the present work.
Objectives

1. Screening and isolation of PGPR from the rhizosphere and roots of a local paddy variety GJ-17 (GR-11).

2. Elucidating the effects of the isolated organisms on the growth and yield of paddy against biotic and abiotic stresses.

3. Identification of factors involved in the plant growth promoting activities through morphological, anatomical, biochemical and molecular analysis.