IndoleAceticAciproductionBy PSEUDOMONAS *SPECIES And Its Effect On Plant Growth Of Green Gram And Black Gram*

By

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Dedicated

To My

Beloved parents

CERTIFICATE – I

This is to certify that this thesis entitled, **"Indole acetic acid production by *Pseudomonas* species and its effect on plant growth of green gram and black gram",** submitted for the degree of **Master of Science** in the subject of **Microbiology** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Mr. Om Prakash** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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This is to certify that this thesis entitled, **"Indole acetic acid production by *Pseudomonas* species and its effect on plant growth of green gram and black gram"**, submitted by **Mr. Om Prakash** to the Chaudhary Charan Singh Haryana Agricultural University, in partial fulfilment of the requirements for the degree of **Master of Science,** in the subject of **Microbiology**, has been approved by the Student's Advisory Committee after an oral examination on the same.

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2. REVIEW OF LITERATURE

Fluorescent pseudomonads and bacilli comprise a major group among plant growth-promoting rhizobacteria (PGPR) along with other bacteria like *Agrobacterium, Alcaligenes, Azotobacter, Burkholderia, Clostridium, Rhizobium* and *Serratia* (Sindhu *et al*., 1997). Preston (2004) reported that plant-associated *Pseudomonas* lives as saprophytes and parasites on plant surfaces and inside plant tissues. Many plant-associated pseudomonads promote plant growth by suppressing pathogenic microorganisms, synthesizing plant growth-stimulating hormones, increasing the availability and uptake of nutrients and/or by promoting plant disease resistance. Other *Pseudomonas* species inhibit plant growth and cause disease symptoms ranging from rot and necrosis through to development dystrophies such as galls. Pathogenic, saprophytic and plant growth-promoting strains are often found within the same species, and the incidence and severity of *Pseudomonas* diseases are affected by environmental factors and host-specific interactions. Plants are faced with the challenge of how to recognize and exclude pathogens that pose a genuine threat, while tolerating more benign organisms.

2.1 Occurrence of microorganisms in the soil and rhizosphere

The microbial growth and activity is particularly intense in the rhizosphere. The rhizosphere environment is richer in nutrients and its microbial communities differ from those present in soil (Alexander, 1977). It is now recognized that the 'rhizosphere effect' is due to the availability of root exudates which attract soil microorganisms. This is reflected by the number of bacteria and other microbes that are found around the roots of plants and that is generally 10 to 100 times higher than in the bulk soil. The physical and chemical properties of soil also play a dominant role in multiplication and survival of soil microorganisms. The width of the zone of soil influenced by the living plant root varies with the plant species, its age, cultural conditions and environmental factors (Rangaswami and Bagyaraj, 1998). Bacteria are the most dominant group of microorganisms in the soil and contribute about 300 - 3000 kg wet biomass per hectare of soil. The most abundant bacterial forms or genera present in the soil are *Arthrobacter, Bacillus, Burkholderia, Clostridium, Corynebacterium, Enterobacter, Flavobacterium, Micrococcus, Rhizobium, Pseudomonas, Sarcina, Serratia* etc. (Rangaswami and Bagyaraj, 1998; Subba Rao, 2000).

Some bacterial species living in the rhizosphere can effect plant growth in either a positive or in a negative way. Beneficial effects of

rhizosphere bacteria have most often been based on increased plant growth and seedling emergence, enhanced nodulation and nitrogen fixation in leguminous crops and suppression of diseases. As a consequence PGPR have been further divided into subsets like emergence promoting rhizobacteria (EPR), nodulation promoting rhizobacteria (NPR) and disease suppressing rhizobacteria (DSR) (Kloepper *et al*., 1986). For instance, PGPR that act through biological control can also enhance germination or plant growth. In recent years, many reviews have appeared dealing with PGPR isolation, screening, ecology, physiology and their use as agro-biotechnological inoculants (Glick, 1995; Lazarovits and Nowak, 1997; Saxena *et al*., 2000; Goel *et al*., 2001a; Dobbelaere *et al*., 2003).

Sorenson *et al.* (2001) reviewed the factors that control the successful performance of *Pseudomonas* spp. as inoculants and its establishment, proliferation and activity under *in situ* conditions. The use of advanced microscopy using fluorescent antibodies and marker gene technology as tracking methods, have provided new insight into the significance of growth limiting factors in the soil and along the root.

2.2 Isolation and identification of PGPR

Bacteria have been isolated from the rhizosphere and rhizoplane zones of different crop plants (Ladha *et al*., 1983; Glick *et al*., 1995; Chabot *et al*., 1996b; de Frietas and Germida, 1997; Sindhu *et al.* 2002). Hellriegel and Wilfarth (1886) demonstrated that the ability of pea plants to convert dinitrogen form the atmosphere into plant utilizable compounds was associated with the presence of swellings or nodules on legume roots. Beijernick (1888) isolated the microorganisms responsible for nodulation of leguminous plants and named as *Bacillus radicicola*, which was subsequently renamed as *Rhizobium*. Subsequently *Rhizobium* strains were isolated from nodules of diverse leguminous plants grown under different agro-climatic regions and their symbiotic effectiveness was studied (Gaur, 1993; Sindhu and Dadarwal, 2000).

Enterobacter and nonspecific bacterial populations were found as a major component of the rhizosphere microflora when isolations were made from the soybean rhizosphere using the dilution plate method. Gram-negative bacteria and actinomycetes were next in abundance, followed by pseudomonads (Hicks and Loynachan, 1989). A total of 300 isolates were obtained from the rhizosphere of 9-month old winter wheat plants (cv. Norstar) using (i) combined carbon (CC), (ii) Mac Conkey's (MAC) and MAC plus 100 µg ml^{-1} carbenicillin (MACC), and (iii) trypticase soy agar (TSA)

medium. These were further screened for their ability to grow on King's B medium (KB) and KB medium supplemented with antibiotics (chloramphenicol, cycloheximide, novobiocin and penicillin) to make it selective for fluorescent pseudomonads (de Freitas and Germida, 1990).

Fages and Arsac (1991) isolated forty five gram negative bacterial strains from the rhizosphere of sunflower and these isolates were screened for their growth promoting activity Malik *et al*., (1995) isolated the growth-promoting rhizobacteria namely *Klebsiella planticola, Proteus vulgaris* and *Bacillus subtilis* from root region and root surfaces of sunflower.

A rapid and novel procedure for the isolation of PGPR has been described by Glick *et al*. (1995). This method entails screening of soil bacteria for the ability to utilize the compound 1 - aminocyclopropane -1-carboxylate (ACC) as a sole N source, a trait that is a consequence of the presence of the enzyme ACC deaminase activity. This trait appears to be limited to soil bacteria that are capable of stimulating plant growth.

Khot *et al.* (1996) isolated 36 rhizobacteria from rhizosphere of chickpea plants using fungal cell wall material as carbon source for growth. Five bacteria were found to inhibit the growth of *Fusarium oxysporum* and *Rhizoctonia bataticola*. Gupta *et al.* (1998a) isolated rhizobacteria from the

rhizotic zones of green gram using 7 selective and 4 non-selective media. A total of 121 bacteria were isolated of which Gram-ve bacteria accounted for 65%. The dominant genera were *Pseudomonas, Bacillus, Enterobacter, Proteus* and *Klebsiella.*

Cattelan *et al*. (1998) isolated 1131 bacteria from bulk soil and the rhizosphere of soybean. On the basis of fatty acid methyl ester (FAME) identification, 60 isolates representing 15 different species from eight different genera were selected. The most common genera were *Pseudomonas, Burkholderia, Bacillus* and *Alcaligenes*.

de Frietas and Germida (1997) isolated phosphate-solubilizing bacteria belonging to *Bacillus brevis, B. megaterium, B. polymyxa, B. sphaericus, B. thuringiensis* and *Xanthomonas maltophilia*. Employing the semisolid nitrogen free media, a total of 285 strains of *Azospirillum* were isolated from soils of seven geographic regions in New South Wales, Australia (Han and New, 1998). By combining amplification and restriction analysis of 16S rDNA patterns with serological, morphological and biochemical results, all isolates were found to be *A. brasilense* and *A. lipoferum.*

2.3 Mechanisms of plant growth promotion

PGPR promote plant growth by various mechanisms that include : (i) the ability to produce or change the concentration of the plant hormones like indole acetic acid (Mordukhova *et al*., 1991), gibberellic acid (Mahmoud *et al*., 1984), cytokinins (Tien *et al*., 1979) and ethylene (Arshad and Frankenberger, 1991; Glick *et al*., 1995); (ii) asymbiotic N_2 fixation (Boddey and Dobereiner, 1994; Kennedy *et al*., 1997); (iii) solubilization of fixed phosphates and other nutrients (de Freitas *et al*., 1997) and (iv) antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), β-1,3-glucanase (Fridlender *et al*., 1993), chitinases (Renwick *et al*., 1991), antibiotics (Shanahan *et al*., 1992) and cyanide (Flaishman *et al*., 1996).

2.3.1 Antagonism against phytopathogenic fungi

During the last two decades, several rhizobacteria have been reported to provide substantial disease control in the field (Saxena *et al*., 2000). Many bacterial genera have shown their potential for biocontrol both under *in vitro* and *in vivo* conditions. The bacteria involved in suppression of soil borne pathogens include: *Agrobacterium* (Thomson, 1987), *Alcaligenes* (Martinetti and Loper, 1992), *Azotobacter* (Meshram and Jager, 1983), *Bacillus* (Asaka and Shoda, 1996; Silo-Suh *et al*., 1994), *Enterobacter* (Costa and Loper, 1994; Roberts *et al*., 1997a,b), *Pseudomonas* (Kloepper *et al*.,

1980; Hebbar *et al*., 1992; Hallman *et al*., 1996), *Burkholderia* (King and Parke, 1993), *Rhizobium* and *Bradyrhizobium* (Chakraborty and Purkayastha, 1984), *Serratia* and *Stenotrophomonas* (Berg and Behl, 1997). Many of these biocontrol agents exhibited their effectiveness in disease control under field conditions also.

Fluorescent pseudomonads have emerged as the most promising group of plant growth-promoting rhizobacteria involved in the biocontrol of plant diseases during the last 20 years (Burr *et al*., 1978; Pierson and Weller, 1994; Wei *et al*., 1996). These fluorescent *Pseudomonas* spp. have been implicated in the control of several wilt diseases caused by *Fusarium* spp. (Hebbar *et al*., 1992; Chen *et al*., 1995); root rot of important crops like wheat, cucumber and tulip caused by *Pythium* spp. (Paulitz *et al*., 1992; Moulin *et al*., 1996), take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* (Pierson and Weller, 1994; Duffy *et al*., 1996) and several other fungal diseases like grey mould caused by *Botrytis cinerea* (Walker *et al*., 1996) and sorghum anthracnose caused by *Colletotrichum graminicola* (Michereff *et al*., 1994a). Numerous mechanisms for protection have been proposed including production of antibiotics, siderophores, and hydrolytic enzymes, accumulation of antifungal metabolites, nutrient competition and niche exclusion (Chen *et al*., 1995; Goel *et al*. 2001a).

The usefulness of *Bacillus* an antagonist for many plant pathogens is well known (Campbell, 1989). *Bacillus* has ecological advantages because it produces endospores that are tolerant to extreme environmental conditions such as heat and desiccation. Broadbent *et al*. (1971) isolated *Bacillus subtilis* strain A13 from lysed mycelium of *Sclerotium rolfsii*. It was found to be antagonistic towards several pathogens and its use as seed inoculant increased the yield of carrots by 48%, oats by 33% (Merriman *et al*., 1974) and peanuts by 37% (Turner, 1987). Since 1983, it has been sold as a bioinoculant under the trade name QUANTUM-4000 (Turner, 1987). *Bacillus cereus* UW85 was found to protect alfalfa seedlings from damping-off caused by *Phytophthora medicaginis* (Handelsman *et al*., 1990), tobacco seedlings from *Phytophthora parasitica* var. *nicotianae* (Handelsman *et al*., 1991), cucumber fruits from *Pythium aphanidermatum* (Smith *et al*., 1993), and peanut from *Sclerotinia minor* blight (Phipps, 1992). The suppression of alfalfa damping-off disease was attributed to the production of two fungistatic antibiotics (Silo-Suh *et al*., 1994). Likewise, *Bacillus subtilis* RB14, produced antibiotics iturin and surfactin that suppressed damping-off disease of tomato caused by *Rhizoctonia solani* (Asaka and Shoda, 1996).

Basha and Ulaganathan (2002) isolated a soil bacterium, *Bacillus* sp. strain BC121, from the rhizosphere of sorghum. It showed high antagonistic

activity against *Curvularia lunata*. A clear inhibition zone of 0.5 - 1.0 cm was observed in dual plate assay. Scanning electron microscopic observations showed a clear hyphal lysis and degradation of fungal cell wall. In dual cultures, the *Bacillus* strain BC121 inhibited the *C. lunata* up to 60% in terms of dry weight. This strain also produced a clear halo region on chitin agar medium plates containing 0.5% colloidal chitin, indicating that it excreted chitinase. The protein extract from the wild strain, when tested on SDS-PAGE gel showed a unique band corresponding to the molecular mass of 25 kDa, which could be the probable chitinase protein.

2.3.2 Asymbiotic nitrogen fixation

Root-associated nitrogen-fixing bacteria are found in diverse soils which vary widely in nitrogen content. The capability to fix N_2 provides a strong competitive advantage in soils with low organic nitrogen content. Long-term nitrogen-balance studies have shown these microorganisms to be capable of fixing and accumulating significant rates of nitrogen (Day *et al*., 1975; Neyra and Dobereiner, 1977). Several studies indicate N_2 fixation rates of about 30-40 kg ha^{-1} by associative bacteria.

Sprent (1979) presented an extensive list of species currently known to fix nitrogen. Common genera capable of fixing nitrogen include *Azospirillum,*

Azotobacter, Bacillus, Clostridium, Derxia and *Klebsiella*. These are commonly designated as "free-living" bacteria, since they are able to exist in the soil and reduce nitrogen without entering into symbiotic association with plants. Significant N_2 fixation by free-living diazotrophs has been demonstrated in the rhizosphere and on the surface of roots (Boddey and Dobereiner, 1994; Okon and Labandera-Gonzalez, 1994). By employing a variety of methodologies such as the acetylene reduction assay and the ^{15}N dilution technique (*in situ* and in the field), it has been estimated that associative symbiosis could contribute to about 5 kg N ha^{-1} in *Azospirillum* inoculated wheat, sorghum and maize fields (Boddey and Dobereiner, 1994; Okon and Labandera-Gonzalez, 1994).

It has been reported that in some cultivars of sugarcane in Brazil and of kallar grass in waterlogged salty soils in Pakistan, substantial quantities of nitrogen (about 50 kg N ha^{-1}) can be fixed by free-living diazotrophs *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae.* These N_2-fixing bacterial endophytes might be responsible for the proposed high N_2 fixation in sugarcane (Boddey and Dobereiner, 1994).

2.3.3 Solubilization of mineral phosphates and other nutrients

A majority of agricultural soils contain large reserves of phosphorus, of which a considerable part is accumulated as a consequence of regular applications of P-fertilizer (Richardson, 1994). The phenomenon of fixation and precipitation of P in soil is dependent on pH which can reduce efficiency of soluble P fertilizers (Goldstein, 1986). In acidic soils, P is precipitated as Al and Fe phosphates, whereas in calcareous soils high concentration of Ca results in P precipitation. The diverse groups of organisms in soil employ a variety of solubilization reactions to release soluble phosphorus from insoluble phosphates (Salih *et al*., 1989; Illmer and Schinner, 1995; Singh and Kapoor, 1998). The potential of these phosphate solubilizing microorganisms (PSM) have been utilised as bioinoculants for crops grown in soils low in available P and amended with rock phosphate or tricalcium phosphate (Chabot *et al*., 1996a; de Freitas *et al*., 1997; Bagyaraj *et al*., 2000).

In growth chamber studies, inoculation of *Bacillus circulans* and *Bacillus megaterium* var. *phosphaticum* increased plant weight and P-uptake of millet and pea, respectively (Saber *et al*., 1977; Raj *et al*., 1981). Datta *et al*. (1982) isolated a P-solubilizing and IAA producing strain of *B. firmus* that increased the grain yield and P-uptake of rice grown in P-deficient soil amended with rock phosphate. Gaind and Guar (1991) reported increased biomass, grain yield, P and N uptake by mungbean inoculated with *B.*

subtilis. Several phosphate-solubilizing strains belonging to genera *Bacillus* and *Xanthomonas* were found to enhance the growth and yield of canola (*Brassica napus* L.) but did not influence the P-uptake by plant (de Freitas *et al*., 1997). The production of IAA-like hormones by these rhizobacteria was suggested to influence the growth and yield of plant. Rhizobia have also been shown to solubilize organic phosphates (Abed-Alla, 1994). Chabot *et al*. (1996a) reported increase in dry matter and P-uptake of maize and lettuce by inoculation with two strains of *Rhizobium leguminosarum* biovar *phaseoli* (P31 and R1).

2.3.4 Production of plant growth regulating substances by PGPR

The capacity to produce indole-acetic acid (IAA) is wide spread among plant-associated bacteria (Gaudin *et al*., 1994; Patten and Glick, 1996; Glickmann *et al*., 1998). Estimates of the number of IAA-producing organisms range as high as 80% of total soil bacteria highlighting the enormous contribution potential of these organisms to plant's endogenous pool of IAA. The IAA production ability may allow bacteria to detoxify excess tryptophan or tryptophan analogs that are deleterious to bacterial cell, as some IAA biosynthetic enzymes can convert methylated and halogenated substrates to less toxic compounds (Hutcheson and Kosuge, 1985; Yamada *et al*., 1985; Bar and Okon, 1992). IAA is also suggested to regulate the expression of

important compounds in bacteria such as cAMP and amino acids (Katsy, 1997). Perhaps the most obvious utilization for the prevalence of bacterial production of phytohormones is that it may increase production of plant metabolites that bacteria can utilize for their own growth. Another consequence of enhanced plant growth is production of greater root surface area through which more of these metabolites can be exuded (Gaudin *et al*., 1994).

Omay *et al*. (1993) showed that in liquid culture of *Azospirillum brasilense* Cd, the concentration of IAA increased rapidly with the beginning of stationary phase. This suggested that the increase in IAA production in stationary phase is due to the expression of an overall change in cell metabolism when the carbon source is exhausted. *Bacillus* spp. isolates obtained from the rhizosphere of *Phaseolus vulgaris* 'contender' produced significant amount of IAA when grown in a liquid culture medium supplemented with 100 µg L-tryptophan L^{-1} (Srinivasan *et al*., 1996). In the absence of L-tryptophan, less IAA was produced. The amount of IAA produced by various strains varied from 0.40 to 4.0 µg ml^{-1}.

2.4 Genetic manipulation of PGPR for altered level of IAA production

To understand the influence of IAA on plant growth, mutants could be isolated that either overproduce or underproduce the IAA. IAA underproducing mutants have been isolated in *P. savastanoi* using acridine orange (Comai and Kosuge, 1980) and from *A. tumefaciens* by heat and ethidium bromide treatment (Liu *et al*., 1982). In rhizobacteria where IAA genes were located on the chromosome, insertion of transposon interrupted IAA biosynthesis genes in *P. syringae* pv. *syringae* (Mazzola and White, 1994) and in *A. lipoferum* (Abdel-Salam and Klingmuller, 1987).

Comai and Kosuge (1980) showed the involvement of plasmid DNA in indole acetic acid synthesis in *Pseudomonas savastanoi*. The capability of producing IAA is lost at high frequency upon treatment of the bacterial cells with acridine orange. Acridine orange treatment resulted in loss of plasmid having a molecular weight of $34x10^6$. The reintroduction of this plasmid by transformation into mutants lacking IAA production restored the capability of IAA synthesis. Dubeikovsky *et al*. (1993) reported overproduction of IAA using recombinant plasmids for transformation of *P. fluorescens* with multiple copies of IAA biosynthesis genes from *P. syringae* pv. *savastanoi*.

Xie *et al*. (1996) used transposon mutagenesis to obtain a derivative of *P. putida* GR12-2, designated as *P. putida* GR12-2/aux 1, that secreted approximately four times more IAA than the parental strain. The elevated

IAA levels were a consequence of transposon insertion into a region of the bacterial chromosome that elevated the expression of IAA biosynthesis genes, either directly or by overproduction of tryptophan.

Mazzola and White (1994) reported that homologs of the genes for indole-3-acetic acid (IAA) biosynthesis were retrieved from a genomic library of *P. syringae* pv*. Syringae.* Production of IAA was undetectable in mutant strain Y30-53.29, which was generated by transposition of Tn5 into the *iaaM* gene of *P. syringae* pv*. syringae* Y30. The IAA-deficient (IAA^-) mutant retained the ability to colonize the bean phylloplane and induced disease symptoms on bean which were similar to those produced by the parental strain. Syringomycin biosynthesis by the IAA^- strain was diminished in comparison with parental strain or the mutant genetically restored for IAA production. The results indicated that bacterially-derived IAA, or its biosynthesis, is involved in the regulation of *in planta* growth and in the expression of other factors that affect the host-pathogen interactions.

Suzuki *et al.* (2003) isolated IAA low-producing spontaneous mutant HP72LI after several repeated subculturing of *P. fluorescens* HP72. The IAA low production of the strain HP72LI was due to the low tryptophan side chain oxidase (TSO) activity. The colonization ability of strain HP72 on the bentgrass root is higher than that of strain HP72LI. Moreover, colonization

of strain HP72 on the bentgrass root induced root growth reduction, while strain HP72LI did not induce such growth reduction. The results suggested that IAA production by strain HP72 may play a role in the construction of short root systems and take advantage of root colonization, but does not contribute to the biocontrol properties of *P. fluorescens* HP72.

2.5 Effect of PGPR on root system development

Loper and Schroth (1986) found that two bacterial strains belonging to Enterobacteriaceae were capable of producing copious amount of IAA, leading to reduced plant root elongation and an increased shoot/root ratio in sugarbeet, when applied as seed inoculants. A significant linear relationship was observed between IAA accumulation of the rhizobacterial strains and decreased root elongation, and increased shoot/root ratios of the sugarbeet seedlings. *P. syringae* pv *savastanoi* strain active in IAA production also caused a significant decrease in root elongation and increase in shoot/root ratios whereas strains deficient in IAA production had no effect.

Holl *et al*. (1988) investigated the effect of inoculation with *Bacillus polymyxa* (a soil diazotroph) on crested wheat grass, perennial rye grass and white clover. Plant growth responses (root and shoot dry weights,

root/shoot ratios, and seedling emergence) varied from slightly negative to positive. It was concluded that production of plant growth-promoting compounds similar to IAA by the bacterium were the likely stimuli for the observed increase in plant productivity.

Oberhansli *et al*. (1990) found that a wild type strain of *P. fluorescens* produced ten-fold more IAA than its mutant in presence of 1 mM L-tryptophan. When wheat plants, grown in the artificial soil (pH 6.2) and fertilized with ammonia nitrogen, were inoculated with both strains, a significant increase in the shoot/root ratio was caused by the wild type strain than the mutant strain.

Astrom *et al*. (1993) reported that treatment of wheat seedlings with a cell free culture filtrate of *P. fluorescens* had a strong inhibitory effect on root elongation. However, after detecting IAA production by the bacterium, they concluded that the formation of IAA by the bacterium was too low (0.78±0.09 µg ml^{-1}) to contribute to the inhibitory effect induced in plants. Moreover, a rhizobacterial strain (*Serratia liquifaciens*) with no effect on plant growth produced more IAA (1.50±0.10 µg ml^{-1}).

Ethylene production by PGPR has also been demonstrated as one of the mechanisms of plant growth promotion. A small number of soil bacteria

contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. This enzyme hydrolyzes ACC, the immediate precursor of ethylene in plants to yield ammonium and α-ketobutyrate. Bacteria possessing ACC-deaminase activity may therefore, grow using ammonium as the sole nitrogen source. The beneficial rhizobacteria *Pseudomonas putida* strain GR 12-2 was found to stimulate root elongation of different plants. Later, it was found that this bacterium contains ACC-deaminase (Jacobson *et al*., 1994). Mutants of this strain lacking ACC-deaminase activity did not promote root elongation of canola seedlings, implicating this enzyme in the mechanism of root growth promotion by this bacterium (Glick *et al*., 1994). It was proposed that after binding of the bacteria to the seed coat, ACC is hydrolyzed by ACC deaminase. Thus lowering the level of ethylene during imbibition and then promoting root elongation.

Patten and Glick (1996) showed that root growth promotion by free-living PGPR e.g. *Alcaligenes faecalis, Enterobacter cloaceae, Acetobacter diazotrophicus*, species of *Azospirillum, Pseudomonas* and *Xanthomonas* as well as symbionts such as *Bradyrhizobium japonicum* and *Rhizobium* spp, was related to low level of IAA secretion. In contrast, the inhibitory effect of some deleterious rhizobacteria (DRB) was related to their high amount of IAA excretion e.g. *Enterobacter taylorae* (Sarwar and Kremer, 1995).

Xie *et al*. (1996) suggested that IAA synthesizing PGPR may promote elongation of primary roots. Inoculation of canola seeds with *Pseudomonas putida* GR12-2, which produced low level of IAA, resulted in two to four-fold increase in length of seedling roots whereas treatment of plant roots with an IAA-overproducing mutant significantly inhibited the growth of canola roots.

Malik *et al*. (1997) observed IAA production in 5 PGPR strains. These PGPR when inoculated in kallar grass and rice resulted in increased acetylene reduction activity (ARA) but no correlation was found between IAA production and ARA. Lazarovits and Nowak (1997) observed a 60% increase in cytokinin content of potato plant when inoculated with *Pseudomonas* strain PsJN.

Yeole and Dube (1997) studied the seed bacterization effects with 6 fluorescent pseudomonads on growth and yield of chilli, cotton, groundnut and soybean. Significant increase in seed germination, shoot height, root length, fresh and dry plant weight, and yield of all the crops was observed. The effect was maximum in groundnut followed by soybean, cotton and chilli. Mayak *et al*. (1999) observed the effect of wild type *P. putida* and mutant in the rooting of mungbean cuttings. PGPR treated cuttings had a significant higher number of roots compared with cuttings rooted in water.

Ethylene was involved in the initiation and elongation of adventitious roots in mungbean cuttings.

2.6 Effect of PGPR on nodulation by *Rhizobium/ Bradyrhizobium* strains

Rhizobia form N_2-fixing symbiosis with leguminous crop plants and has been successfully used worldwide as an inoculant. The *Rhizobium* alone is fixing about 120 million tonnes of atmospheric nitrogen in different legumes. Coinoculation of legumes with *Rhizobium* and free-living IAA-producers such as *Azospirillum brasilense*, several species of *Bacillus* and *Pseudomonas* have been found to significantly increase the number of nodules, nodule fresh weight and nitrogenase activity compared to inoculation with *Rhizobium/Bradyrhizobium* alone (Yahalom *et al*., 1990; Malik, 2002; Sindhu *et al*., 1999, 2002). In contrast, similar experiments using mutants of *Bacillus megaterium* with altered IAA production levels (overproducers and underproducers) had a negative effect on these parameters (Srinivasan *et al*., 1996).

2.6.1 Nodulation process in legume plants

The basic process of nodulation can be arbitrarily divided into stages of nodule initiation, nodule invasion and bacteroid maturation followed by

nodule senescence. In the absence of the host, free-living rhizobia are in their saprophytic phase. The legume host roots excrete specific compounds and flavonoids that induce active chemotaxis towards defined regions of the roots. The rhizobia attach to root hairs all over the root. In the infectible zone of roots, rhizobia attach to the root hair surface. The nodulation genes of infecting rhizobia after induction by flavonoids produce Nod factors that cause characteristic curling and deformations of root hair and cortical cell divisions in compatible host.

The rhizobia invade the root hair cell at the root hair tip by means of an infection thread. The rhizobia travel down to the root hair to the underlying cortical cell layers through the inwardly growing tubular tunnel-like infection thread. It proceeds towards the newly developed primordium, formed by induction of mitotic activity in the root cortex due to *Rhizobium*-made diffusible Nod factors and subsequently develops into the nodule meristem. Bacterial invasion spreads from cell to cell by the continued growth of infection threads. In the root cortex, infection threads ramify and penetrate individual nodule cells and the infection droplet, is formed. Rhizobia from such infection droplets are released into nodule tissue cells and then occupy an organelle-like cytoplasmic compartment, termed the “symbiosome”.

Upon release from infection thread, the bacteroids multiply, enlarge and eventually occupy most of the volume of infected cell. In the late symbiotic zone, the infected cells are completely filled with bacteria that have bacteroids. These bacteroids reduce atmospheric nitrogen by the action of the nitrogenase enzyme. Leghaemoglobin protein is synthesized in nodules that help to maintain the low oxygen tension necessary for effective nitrogen fixation. During the programmed senescence of nitrogen-fixing bacteroids, growth and division of bacteroids is stopped and lysis of N_2-fixing bacteroids and the host cells occur.

2.6.2 Stimulation of nodulation by coinoculation of PGPR with *Rhizobium*

Wang *et al*. (1982) reported secretion of IAA in culture medium from both nodulating and non-nodulating strains of *Rhizobium* although levels were low in the absence of tryptophan. With the addition of exogenous tryptophan, IAA production in the nodulating strains of *Rhizobium leguminosarum* was stimulated to a much greater extent than in the non-nodulating mutants. The results suggested that at least two pathways for IAA biosynthesis exist in these strains: one pathway is constitutive and the other is inducible by tryptophan. The inducible pathway that is absent in the non-nodulating strain, may be involved in nodulation.

Kaneshiro and Kwolek (1985) observed nodulation on *Glycine max* L. seedlings by parental *Rhizobium japonicum* (USDA strain 26) and a tryptophan catabolic mutant (tan4b, strain B-14075). Seedlings grown in plastic pouches were compared for increases in root weight, nodule volume and acetylene reduction activity (ARA). The tan4b mutant that produced extracellular indole-3-acetic acid (IAA) and indole-3-pyruvic acid (IPA) enhanced nodulation significantly. Nodulation by strain 26 was most effective when a basal nutrient was supplemented with sucrose, $CaCO_3$, EDTA, nicotinic acid and glutamate. In contrast, effective nodulation by tan 4b did not require an exogenous glutamate (0.34 mM) and nicotinate (81.0 µM) combination.

Bolton *et al*. (1990) studied coinoculation effect of *R. leguminosarum* and a toxin-producing *Pseudomonas* sp. (inhibitory to growth of winter wheat root and of several microorganisms) in pea roots under leonard jar conditions. *Pseudomonas* sp. was found to be rapid colonizer of pea roots and increased the number of nodules formed by *R. leguminosarum.* However, nodule dry weight and pea shoot biomass were similar to plants inoculated with *R. leguminosarum* only.

Parmar and Dadarwal (1999) showed that coinoculation of the rhizobacteria with effective *Mesorhizobium* strains of chickpea resulted in a

significant increase in nodule weight, root and shoot biomass and total plant nitrogen in chickpea. The application of ethyl acetate extracts of culture supernatant fluids when applied to seeds resulted in enhancement of flavonoids in chickpea roots, suggesting that the rhizobacteria have a direct influence on production of flavonoids in roots which may be an additional factor for promotion of root growth and enhanced nodulation by these bacteria (Parmar and Dadarwal, 1999).

Yahalom *et al*. (1987) reported that coinoculation of legumes with *Rhizobium* and free-living IAA producers such as *Azospirillum brasilense*, and several *Bacillus* species significantly increased nodule fresh weight and nitrogenase activity compared to inoculation with *Rhizobium* alone. The combined inoculation of white clover with *R. leguminosarum* bv. *trifolli* and *A. lipoferum* enhanced the number of nodules by 2-3 times and acetylene reduction activity by 2.3 to 2.7 times (Tchebotar *et al*. 1998). In contrast, similar experiments using mutants of *Bacillus megaterium* with altered IAA production level (overproducers and underproducers) had a negative effect on these symbiotic parameters (Srinivasan *et al*., 1996).

Hunter (1994) isolated a mutant of *Bradyrhizobium japonicum* strain that accumulated anthranelic acid, indole, 3-indole-acetic acid, 3-indole-lactic acid and 3-indole-pyrivic acid in culture medium, indicative of altered

tryptophan metabolism. Soybean plants inoculated with these bacteria formed more nodules and showed more nodule mass than the plants inoculated with the wild-type strain.

Fukuhara *et al*. (1994) reported that mutants of *Bradyrhizobium elkanii* deficient in IAA production induced fewer nodules on soybean roots than did the parental strain. The normal number of nodules was re-established by application of exogenous IAA. IAA derived from *B. elkanii* had been implicated as a causative agent in the swelling of outer cortical cells of soybean roots that sometimes accompanies nodule formation and may provide competitive advantage for nodulation (Yuhanshi *et al*., 1995). Enlargement of cortical cells was not observed after inoculation with IAA^- deficient mutants of *B. elkanii* (Yuhanshi *et al*., 1995).

Dashti *et al*. (1997) reported that coinoculation of soybean with *B. japonicum* and *Serratia liquefaciens* increased grain yield, protein yield and total plant protein compared to non-treated control in fumigated and non-fumigated soil. PGPR applied to the rhizosphere without addition of *B. japonicum* increased only leaf area and seed number at one fumigated site. Further, they found that coinoculation of soybean with *B. japonicum* and PGPR increased nodulation and hastened the onset of nitrogen fixation. PGPR also increased total fixed N, protein content and soybean yield..

Sindhu *et al*. (1999) reported that *Pseudomonas* sp. isolated from green gram rhizosphere inhibited a wide range of phytopathogenic fungi. On coinoculation of *Pseudomonas* sp. with *Bradyrhizobium* sp. *Vigna* strain S24 on green gram, a significant increase in nodule number, nodule fresh weight, plant dry weight and total plant N was observed.

2.7. Effect of PGPR on plant growth and yield

There is an enormous literature on the application of PGPR as bioinoculants for improvement of plant growth and yield (Saxena and Tilak, 1994; Okon *et al*., 1998; Goel *et al*., 2001a; Hafeez *et al.* 2004), but few bacteria like *Rhizobium, Azotobacter* and *Azospirillum* have been developed as commercial inoculants. Bacteria belonging to the genera *Pseudomonas* and *Bacillus* species are under maximum scrutiny for potential use in agriculture (Powell and Rhodes, 1994).

Increases in plant height as well as root and shoot biomass of wheat were reported following inoculation with 12 different isolates of PGPR belonging to *Pseudomonas aeruginosa, P. cepacia, P. fluorescens* and *P. putida* (de Freitas and Germida, 1990). Similarly, treatment of wheat seeds with fluorescent pseudomonads (antagonistic to *Gaeumannomyces graminis*) resulted in yield increases of 27% in field trials (Weller and Cook, 1986).

Three PGPR strains namely, *Burkholderia cepacia* strain PHP7, *Pseudomonas fluorescens* strain A23/T3c and *Enterobacter* sp. strain BB23/T4d were tested in combination or alone for growth promotion of *Sorghum bicolor*. All three strains were able to colonize the root system of sorghum but only *B. cepacia* and *P. fluorescens* promoted plant growth in single strain inoculation tests. Dual strain inocula were no more effective than single ones (Chiarini *et al*., 1998).

Rhizobium, a symbiotic nitrogen fixer has also been shown to act as a plant growth-promoting rhizobacteria for non-leguminous crops. Reports indicated that non-legumes respond to the presence of bradyrhizobia and rhizobia in the rhizosphere. Root hair curling induced by these symbiotic bacteria was observed on maize, rice and oat plants (Plazinski and Rolfe, 1985; Terouchi and Syono, 1990). Studies also showed that Nod-factors produced by *Bradyrhizobium* and *Rhizobium*, can be perceived by tomato, as indicated by the induction of alkalization in tomato cell cultures. Noel *et al*. (1996) observed a direct growth promotion of early seedling root of canola and lettuce by *R. leguminosarum* under gnotobiotic conditions. Chabot *et al*. (1996a) observed that phosphate-solubilizing strains of *R. leguminosarum* bv *phaseoli* improved the growth of maize and lettuce. Hotlich *et al*. (1994) obtained significant shoot dry matter yield increases (7-8%) by inoculating

maize, spring wheat and spring barley (*Hordeum vulgare* L.) with *R. leguminosarum* bv. *trifolii* strain R39 in field experiments. Yanni *et al*. (1995) reported that certain effective wild-type strains of *R. leguminosarum* bv. *trifolii* established natural plant-bacterial associations that have the potential to promote rice growth under both field and laboratory conditions. Antoun *et al*. (1998) showed increase in the dry matter yield of radish due to inoculation of *B. japonicum* strain Tal 629.

Coinoculation of PGPR strains with *Rhizobium* or *Bradyrhizobium* strains has been shown to influence nodulation, nitrogen fixation and growth of leguminous plants. Chanway *et al*. (1989) tested nine PGPR strains on a single cultivar of lentil and pea in the field. None of the strains stimulated the growth of pea, but in lentil plots inoculated with one or more rhizobacterial strains, there were significant increase in emergence, vigour, nodulation, C_2H_2 reduction activity and root weight. Turner and Backman (1991) observed that treatment of peanut seeds with *Bacillus subtilis* resulted in improved germination and emergence, enhanced nodulation by *Rhizobium* spp., enhanced plant nutrition, reduced levels of root cankers caused by *Rhizoctonia solani* AG-4 and increased root growth. Podile (1995) found that seed bacterization of pigeonpea with *Bacillus subtilis* AF1 enhanced the percentage emergence, nodulation and plant growth.

IAA producing *Bacillus* isolates promoted root growth and nodulation when coinoculated with *Rhizobium etli* on *Phaseolus vulgaris* in growth chambers (Srinivasan *et al*., 1996). Similarly, coinoculation of soybean with *B. japonicum* and *Serratia liquefaciens* 2-68 or *S. proteamaculans* 1-102 increased soybean grain and protein yield compared to the non-treated controls (Dashti *et al*., 1997, 1998). Gupta *et al*. (1998a) isolated 16 strains of PGPR from rhizosphere zone of green gram which were identified as *Enterobacter, Pseudomonas* and *Bacillus* spp. These PGPR were found to increase shoot and root length, and biomass of green gram plants. *Enterobacter* isolate EG-ER-2 increased the nodule occupancy of *Bradyrhizobium* spp. strain S24 on green gram and another isolate EG-ER-1 promoted nodulation for rhizobial strain COG 15 (Gupta *et al*., 1998b). The coinoculation of PGPR with *Bradyrhizobium* strain showed 60-80% increase in nodule occupancy and increased shoot biomass, N-contents and grain yield of green gram plant.

Savithiry and Ganamanickam (1987) reported that bacterization of peanuts with *P. fluorescens* resulted in taller plants (by 25.7%) and increased the yields by 59.0%. Polonenko *et al*. (1987) reported nodule-promoting effect in soybean by root colonizing becteria *P. fluorescens* and *P. putida* species. All strains colonized soybean roots at level of log 3.9 to 5.7 cfu g^{-1}

roots. They found that root colonizing bacteria generally did not interfere with ability of *B. japonicum* to form nodules in soybean roots, rather, certain strains were found to enhance nodulation and plant growth.

Knight and Langston-Unkefer (1988) observed an approximate doubling in plant growth, total plant nitrogen, nodulation and dinitrogen fixation in alfalfa as a result of root colonization by a toxin-releasing pathogen *P. syringae* which produces tabtoxinine β-lactum (an irreversible inhibitor of glutamine synthetase).

Zhang *et al*. (1997) studied coinoculation effect of 9 rhizobacterial strains with *B. japonicum* 532 on soybean nodulation and nitrogen fixation at three root zone temperatures (RZT). At all the temperatures, PGPR strains were found to increase the number of nodules formed and the amount of fixed nitrogen on coinoculation with *B. japonicum*. However, the effect varied with temperature. Enhancements of plant physiological activities were detected before the onset of nitrogen fixation. Shabaev *et al*. (1998) observed an increase in the weight of grains and above ground parts of soybean in a microplot experiment on a grey forest soil on coinoculation of *B. japonicum* 110 with *P. fluorescens* 20 or *P. fluorescens* 21 and *Glomus mosseae*. It was accompanied by an increase in amount of 'biological' nitrogen and 'starting' mineral nitrogen in the yield.

Kumar (1999) reported that bacterization of chickpea seeds with a siderophore-producing fluorescent *Pseudomonas* strain RBT13 (isolated from tomato rhizoplane) increased shoot height, root length, fresh weight, dry weight and yield in soil infested with *F. oxysporum* f. sp. *ciceri*. Rao *et al*. (1999) isolated 5 strains of fluorescent *Pseudomonas* from the rhizoplane and rhizosphere of pea and soybean which exhibited both growth promoting and biocontrol activities. Bacterization of lentil with these isolates resulted in improved seed germination, improved fresh root and shoot weight of the plants and also improved nodulation.

Goel *et al*. (2000) obtained a nonpigment-producing mutant (MRS16-M5) from a pigment-producing *Pseudomonas* sp. MRS16. The mutant as well as the parent when used as inoculant for chickpea, showed initial root stunting effect followed by root proliferation. Coinoculation of chickpea with *Pseudomonas* strain MRS16 as well as MRS16-M5 with *Rhizobium* sp. *Cicer* strain Ca181 enhanced nodulation, nitrogen fixation and plant dry mass as compared to single inoculation with *Rhizobium* strain. In coinoculation experiments, effect of the nonpigment-producing mutant was at par with pigment-producing parent strain.

Dileep Kumar *et al*. (2001) showed that seed bacterization with 5 plant growth promoting fluorescent *Pseudomonas* strains and 3 *Rhizobium*

leguminosarum biovar *viceae* strains promoted the plant growth in *Pisum sativum* L.cv. Capella. Coinoculation of the fluorescent pseudomonads and *Rhizobium* improved plant growth in terms of shoot height, root length and dry weight. Seed bacterization with plant growth-promoting strains alone and together with a rhizobial isolate, R361-27 reduced the number of infected peas grown in *Fusarium oxysporum* infested soils. In a synthetic culture medium, all the plant growth-promoting fluorescent pseudomonad strains produced siderophores, which showed antifungal and antibacterial activities.

Devanand *et al*. (2002) conducted a field experiment under rainfed conditions to study the effect of plant growth promoting rhizobacteria on growth and yield of pigeon pea cultivars ICPL-87 and ICPL-87119. The seeds were inoculated with *Rhizobium* sp. (GB-1), *Azospirillum* sp. (ACD-20) and *Pseudomonas striata* (strain 27), alone or in combination, prior to sowing. A significant increase was observed in plant height, nodule number, nodule dry weight, dry matter production, yield and 100-seed weight in plants inoculated with *Rhizobium* sp. + *Azospirillum sp*. + *P*. *striata*.

Goel *et al*. (2002) reported that two *Pseudomonas* strains MRS23 and CRP55b showed antagonistic activity towards the pathogenic fungi *Aspergillus* sp., *Fusarium oxysporum* f. sp. *ciceris*, *Pythium aphanidermatum*

and *Rhizoctonia* solani under culture conditions. Both the strains produced siderophores in agar plates as well as in liquid cultures. *Pseudomonas* strain MRS23 was also found to produce hydrocyanic acid (HCN). Seed bacterization with *Pseudomonas* strains of two chickpea (*Cicer arietinum*) cultivars, H8618 and C235, showed root-stunting effect at 5 days, whereas this inhibitory effect was overcome at 10 days of seedling growth in cv. H8618. Coinoculation of chickpea with *Pseudomonas* strains MRS23 and CRP55b and *Mesorhizobium* sp. *Cicer* strain Ca181 resulted in the formation of 68.2-115.4% more nodules at 80 and 100 days after planting as compared to single inoculation with the *Mesorhizobium* strain under sterile conditions. The shoot dry weight ratios of coinoculated treatments at different stages of plant growth varied from 1.18 to 1.35 times that of *Mesorhizobium*-inoculated and 3.25 to 4.06 times those of uninoculated controls.

Baig *et al*. (2002) isolated a total of 105 bacteria from the rhizosphere and rhizoplane of groundnut. Of these, *Pseudomonas* was the most predominant (42%) followed by *Bacillus* (28%) and *Enterobacter* (21%). The effect of the isolates on plant growth varied; plant showed stunted growth, root and shoot elongation, or a neutral response. Three isolates increased root length while 14 isolates increased shoot length over the uninoculated control. An increase in fresh and dry matter was recorded by 16 strains.

Asghar *et al*. (2004) screened one hundred rhizobacteria previously isolated from the rhizospheres of *Brassica* species for their growth promoting activity in *Brassica napus* L. under gnotobiotic conditions. Results revealed that 58% of the rhizobacteria increased root length (up to 139%), 39% enhanced shoot length (upto 78%), and shoot weight (upto 72%) of *Brassica napus* L. These isolates were then assayed for their ability to produce auxins *in vitro* in the presence and absence of L-tryptophan. Regression analysis showed that *in vitro* auxin production by these bacteria was significantly related to the number of branches and oil content of *B. napus*. Results indicated that simultaneous screening of rhizobacteria for growth promotion under gnotobiotic conditions and *in vitro* production of auxins could be a useful approach for selecting effective PGPR.

Hafeez *et al*. (2004) conducted experiments to determine the growth promoting activities of various rhizobia in cotton (*Gossypium hirsutum* L.) under growth room conditions. Seeds of 4 cotton cultivars were inoculated with four indole-3-acetic acid producing selected (*Brady*) *Rhizobium* strains and *Azotobacter* strains included as a positive control. Growth responses to inoculation exhibited bacterial strain-cotton cultivar specificity and also included increase in rate of seedling emergence by 3-9%. Shoot dry weight, biomass and N uptake were increased by 48, 75 and 57%, respectively, due

to inoculation with both the *Rhizobium leguminosarum* bv. *trifolii* E11 and *Azotobacter* sp. S8, whereas, strain E11 also increased root dry weight, root length and area by 248, 332 and 283%, respectively. K^+ and Ca^{2+} uptake was also increased by 2-21% and 9-14%, respectively, due to rhizobial inoculation. The results showed that (*Brady*) *Rhizobium* strains promoted cotton growth through efficient nutrient uptake, which was mainly related to increased root growth due to the effect of IAA produced by these strains.

Effect of coinoculation of Pseudomonas mutants with Bradyrhizobium strain 524 in green gram under sterile conditions

Seed inoculation of green gram with Bradyrhizobium sp. strain 524 alone or on coinouclation with different mutants derived from Pseudomonas strain CPS59 increased the nodule number and shoot dry weights in comparison to uninocualted controls (Table 9). The shoot dry weight gains varied from 7.5 to 49.4% times those of Bradyrhizobium inoculated treatments and 180.8 to 290.1% those of uninoculated controls at 60 days of plant growth. Six Pseudomonas mutants, at 60 days of plant growth. Six pseudomonas mutants,CPS59-64, CPS59-138, CPSS9-231, CSPS9-149, CPS59-193 and CPS59-311 caused significant gains in shoot dry weight ratios i.e. 1.32, 1.49, 1.43, 1.36, 1.36, 1.34 and 1.38 times those of Bradyrhizobium inoculated plants, respectively. Nodule promoting effect was observed after coinoculation with mutants CPSS9-165, CPS59-138, CPS59-231, CPS59-162 and CPS59-321 at 30 days of growth whereas all the midetns except CPS59-183 enhanced nodule formation at 60 days of growth.

Maximum nodule-promoting effect was observed with mutants CPS59-138, CPS59-193, CPS59-231 and CPS59-321 at 60 days of plant growth. Pseudomonas mutant dependent variations of various treatments at different stage of plant growth.

Coinoculation of mutants derived from Pseudomonas strain MPS 90 also enhanced the shot dry weights in green gram except the mutant MPS90-157 at 30 days of plant growth (Table 10). The shoot dry weight ratio of coinoculated plants over control or Bradyrhizobium inoculated plants varied from _____ to _____, and _____ to _____ times, respectively at 60 days of plant growth. Significant gains in shoot dry weight were observed with mutants MPS90-133, MPS90-14, MPS90-106, MPS90-138 and MPS90-280 also increased nodule number and nodule biomass, indicating stimulation of nodualtion by bradyrhizobia on coinoculation with some of the Pseudomonas mutants.

IAA low producer and over producer mutants were also coinoculated with Bradyrhizobium strain S24 in black gram. The IAA overproducer mutant showed more enhancement in nodule formation in comparison to IAA low producer mutants. Maximum gains in shoot dry weights were observed on coinoculation of Pseudomonas mutants CPS59-64, CPSS9-149 CPS 59-162 and CPS 59-193 at 45 days of growth. Shoot dry weight ratios of coinoculated plants over control plants varied from_____ to _____ whereas only _____ to _____ times increase was observed in comparison to those of Bradyrhzobgium moculated palnts.

Tn 5 derived mutants of Psendomonas strain MPS 90 having altered level of TAA production were alos used for seed inoculation of black grain. Five pseudomonas mutants MPS 90-39, MPS 90-51, (low IAA producer), MPS 90-14, MPS 90-102 (IAA produced equal to parent strains) and MPS 90-280 showed significant gains in shoot dry weights showed significant gains in shoot dry weights at both 30 and 45 days of plant growth. The shoot dry weight gains varied from_____ to _____ times those of Bradyrhizobium-inoculated treatments and _____ to _____ times those of uninocualted controls. Four Pseudomonnas mutants MPS 90-157, MPS 90-14, MPS 90-102 and MPS 90-280 caused more nodule formation by Bradyrhizobium strain 524 in comparison to stimulation by parent Pseudomonas at 30 and 45 days of growth.

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ABSTRACT

Title of the thesis : Indole acetic acid production by *Pseudomonas* species and its effect on plant growth of green gram and black gram

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In the present studies, IAA producing *Pseudomonas* strains CPS59 and MPS90 were mutagenized with transposon Tn5 using E. coli strain S17-1. The frequency of mutants with low amount of IAA production varied from 35.14 to 46.75% from *Pseudomonas* strains CPS59 and MPS90, respectively and only 3.43 to 3.75% mutants produced higher levels of IAA in comparison to respective parent strains in LB medium supplemented with tryptophan. Inoculation of mutants derived from *Pseudomonas* strain CPS59 showed stunting effect on root development of green gram seedlings at both 5 and 10 days of observation whereas mutants CPS59-138, CPS59-162 and CPS59-231 showed stimulatory effect on root growth at 5 days in black gram. Majority of the mutants caused stimulation of shoot growth at both 5 and 10 days of observation in comparison to uninoculated treatment. MPS90-derived mutants i.e. MPS90-39,

MPS90-157, MPS90-102 and MPS90-106 showed slight stimulation of root growth whereas most of the mutants showed stunting effect on shoot growth at 10 days in black gram.

Coinoculation studies of *Pseudomonas* mutants with *Bradyrhizobium* sp. strain S24 resulted in increased nodule number, nodule fresh weight and shoot dry weight in green gram and black gram under chillum jar conditions. Shoot dry weight gains in green gram after coinoculation with CPS59-derived mutants varied from 107-149 per cent and from 110-137 per cent with MPS90-derived mutants in comparison to *Bradyrhizobium*-inoculated plants at 60 days of growth. Similarly, shoot dry weight gains in black gram varied from 102-178 per cent and 105-198 after inoculation with mutants derived from CPS59 and MPS90, respectively. The stimulation effect on shoot dry weight in comparison to uninoculated control treatment varied from 280-390 per cent in green gram and 179-357 per cent in black gram. Four mutants CPS59-138, CPS59-321, MPS90-133 and MPS90-51 showed more enhancements in nodule formation by *Bradyrhizobium* strain S24 in green gram whereas mutants CPS59-162, CPS59-64, MPS90-102 and MPS90-280 caused more stimulation for nodule formation in black gram at 60 days of plant growth. Significant enhancements in shoot dry weights of green gram were observed by coinoculation with *Pseudomonas* mutants CPS59-138, CPS59-231, CPS59-321, MPS90-280, MPS90-14 and MPS90-145. Similarly, coinoculation of *Bradyrhizobium* strain with *Pseudomonas* mutants CPS59-162, CPS59-321, CPS59-214, MPS90-102, MPS90-280 and MPS90-51 showed significant gains in shoot biomass of black gram at 60 days of plant growth. The better performance of IAA over producing mutants in relation to nodulation and plant biomass indicated that IAA production by *Pseudomonas* strains is a beneficial trait for selection of rhizobacterial strains having plant growth promotion ability.

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