7 Interactions Among Beneficial Microorganisms

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7.1 Introduction

The plant rhizosphere is a unique environment characterized by the continuous supply of low molecular weight compounds exuded from the roots. The rhizosphere supports a large and metabolically active microbial population that can be several orders of magnitude higher than in non-rhizosphere soil (Schloter et al. 1997). Interactions between the plant and microorganisms and among rhizosphere microorganisms are largely unknown and recent works show that these interactions are complex and are dependent on multiple traits (Lugtenberg and Dekkers 1999). Although the rhizosphere appears to be too complex to allow its manipulation, specific bacteria can be applied to seed or roots, which cause an alteration in the composition of the rhizosphere. In addition to the manipulation of the microorganisms to discourage disease causing organisms, it should be possible to promote the activity of beneficial ones, such as arbuscular mycorrhizal (AM) fungi, plant growth promoting rhizobacteria (PGPR) and *Rhizobium* sp. Thus the focus of attention has now shifted from plant-microbe interactions to plant-microbe-microbe interactions. Attempts have been made not only to highlight increase in biological activities in two or three membered associations of organisms (Barea et al. 2004) but also to decipher the mechanisms involved in such interactions (Ma et al. 2003). Such syntropic associations are of ecological importance with implied agricultural significance. In the present review, an attempt has been made to discuss interactions among certain groups of beneficial microorganisms like AM fungi, *Rhizobium* sp. and PGPR.

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7.2
Interaction Between *Rhizobium* and PGPR

Rhizospheric bacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Azorhizobium* can interact with roots of legumes to form nodules, which function as sites for atmospheric nitrogen fixation (Relic et al. 1994). Root nodule formation is a complex developmental process involving sequential exchange of chemical signals between the bacterial microsymbiont and the host plant (Long 1996). Both plant and bacterial signals have been identified and shown to play a major role in the specificity of the legume-*Rhizobium* interaction (Downie 1994).

7.2.1
Rhizobium and Nodulation Promoting Rhizobacteria

Specific microorganisms have considerable potential to alter the composition and activity of rhizosphere microflora such as *Rhizobium* (Schroth and Ole Becker 1990). Bacteria that promote nodulation of legumes by rhizobia are referred to as nodulation promoting rhizobacteria (NPR) (Kloepper et al. 1988). These NPR belong to diverse group of microorganisms and include *Azospirillum*, *Azotobacter*, *Pseudomonas* (Villacieros et al. 2003), *Bacillus*, *Streptomyces* (Samac et al. 2003), *Serratia* and *Aeromonas*.

Coinoculation studies with PGPR and *Rhizobium* have been shown to increase root and shoot dry weight, plant vigour, nitrogen fixation and nodule number in various legumes such as alfalfa (Knight and Langston-Unkefer 1988), common bean (Grimes and Mount 1984), green gram (Gupta et al. 1998), pigeon pea (Podile 1995), chickpea (Sindhu et al. 2002), pea (Bolton et al. 1990), soybean (Dashti et al. 1998), cowpea (Agarwal and Tilak 1989), clovers (Burns et al. 1981) and Sesbania (El-Gamai 1992). The beneficial effects of these bacteria have been variously attributed to their ability to produce different compounds including phytohormones (Molla et al. 2001), toxins (Knight and Langston-Unkefer 1988) and antibiotics (Li and Alexander 1990) to suppress deleterious rhizobacteria (Turner and Beckman 1991) or through some other unidentified mechanism (Halverson and Handelsman 1991). Recently it has been shown that the presence of 1-aminocyclopopane-1-carboxylated deaminase (ACC deaminase) enzyme in *Rhizobium leguminosarum* bv. *viceae* enhances the nodulation of *Pisum sativum*, likely by modulating ethylene levels in the plant roots during the early stages of nodule development (Ma et al. 2003). Insertion mutants, with mutations in the rhizobial ACC deaminase gene (acdS) and its regulatory gene, that could not synthesize ACC deaminase showed decreased nodulation efficiency compared to that of the parental strain. In bean plants, nodulation was increased when *Rhizobium* sp. were coinoculated with *Bacillus polymyxa* (Peterson et al. 1996) or *Azospirillum brasilense* (Burdman et al. 1996). Burdman et al. (1996) related *A. brasilense* mediated stimulation in nodulation of common bean to an increased production of flavonoids by the legume host. Presence of rhizobacteria, belonging to fluorescent *Pseudomonas* and *Bacillus*, in the rhizosphere of chickpea enhanced the level of flavonoid-like compounds in the roots, suggesting that the rhizobacteria have a direct influence on root flavonoids which might be an additional factor in nodule promotion by these bacteria (Parmar and Dadarwal 1999). It was shown that coinoculation of *Rhizobium etli* TAL 182 with *Bacillus* sp. induced root hair proliferation in *Phaseolus vulgaris* and enhanced nodulation of *P. vulgaris* by *Rhizobium* (Srinivasan et al. 1996). Coinoculation with both bacterial species also facilitated heterologous nodulation of *Rhizobium* Tal 182 on *Phaseolus acutifolius* (Srinivasan et al. 1996). *Rhizobium etli* is a narrow host range microsymbiont specific for nodulation on *P. vulgaris* whereas *P. acutifolius* belongs to the cowpea cross inoculation group and has a very specialized bradyrhizobial requirement for nodulation (Somasegaran et al. 1991). Heterologous nodulation of *P. acutifolius* by *R. etli* TAL 82 coinoculated with *B. megaterium* S49 implicates the *Bacillus* more directly in the nodulation process. Similar heterologous nodulation on hairy roots of clover plants by a non-specific symbiont *Rhizobium leguminosarum* bv. *viceae* has been reported (Diaz et al. 1995). In contrast, *Bacillus* sp. CECT 450 that increased nodulation on bean when co inoculated with *Bradyrhizobium tropici* CIAT 899 reduced nodulation on soybean when coinoculated with *B. japonicum* USDA 110 (Camacho et al. 2001). Likewise, *Bacillus cereus* UW85 increased nodulation in soybean indirectly by increasing root growth and not stimulating the nodulation process. However the bacterium had little to no positive effects on pea and common bean symbiosis (Vessey and Buss 2002).

Inoculation of soybean crops with effective *B. japonicum* strains singly or in combination with PGPR strains was found to be important for improving and maximizing the plant growth and nitrogen fixation potential of the crop either in soil which lacks indigenous population of *Bradyrhizobium* sp. or in those soils high in indigenous population, but less effective than the introduced bacteria (Kucey 1988). Dual inoculation with a mixture of *B. japonicum* and *Azospirillum brasilense* was superior over single inoculation with *B. japonicum* with regards to nitrogen fixation and dry biomass of soybean (Galal 1997). Molla et al. (2001) found *A. brasilense* to perform better in root growth and nodule development of soybean compared to *A. lipoferum.* Coinoculation of *Serratia proteamaculans* 1–102 and *S. liquefaciens* 2–68 with *B. japonicum* on soybean not only improved nodule number, plant dry weight and fixed nitrogen but also shortened the time for nodule initiation and increased the nodulation rate (Bai et al. 2002).

The influence of rhizospheric bacteria on the competitive ability of the introduced strain is another interesting area where definite conclusions are yet to be made. Singh and Gaur (1995) reported two strains of rhizospheric bacteria that further improves the nodulation as well as competitiveness of an effective strain of chickpea-*Rhizobium*. It was demonstrated that the rhizospheric isolates produce flavonoid like substances which in turn induced nod gene expression. *Enterobacter* isolates EG-ER-2 and KG-ER-1 improved the nodule occupancy of *Bradyrhizobium* strain S 24 and COG 15 respectively (Gupta et al. 1998). Both isolates produced antibiotics and siderophores that might have inhibited other rhizospheric rhizobia enabling the inoculant bradyrhizobial strains to occupy successfully the nodulation sites.

7.2.2

Rhizobium and Biocontrol Agents

Most rhizobacteria used as biocontrol agents have been shown to stimulate legume-*Rhizobium* symbiosis. In contrast several reports suggest inhibition of rhizobia by rhizospheric bacteria on agar plates. Smith and Miller (1974) showed that eight out of nine rhizospheric bacteria inhibited *B. japonicum* on agar plates. Their study also revealed that, as a group, rhizosphere organisms were more inhibitory to *B. japonicum* than non-rhizospheric organisms. In another study, of the 115 rhizospheric rizobacteria tested, 23 inhibited one or more strains of *B. japonicum*. All of the rhizospheric bacteria that inhibited bradyrhizobia produced fluorescent, yellow green, diffusible pigments and were classified as fluorescent *Pseudomonas*. Most of the inhibitory effects of *Pseudomonas* sp. on bradyrhizobia were caused by siderophore-induced iron deprivation (Fuhrmann and Wollum 1989). Three *P. fluorescens* strains UP61, UP143 and UP 148 did not modify the shoot dry weight and rate of nodulation of birdsfoot trefoil, despite antagonistic activity against rhizobia in vitro (De la Fuente et al. 2002). Cattelan et al. (1999) found several rhizospheric isolates which stimulated aspects of soybean-bradyrhizobia symbiosis and which had β-glucanase or cyanide production. These attributes are required for biocontrol of pathogenic fungi but their exact role in stimulation of symbiosis is not clear. In another study inoculation of HCN producing strain of *P. fluorescens* F113rif alone had a deleterious effect on alfalfa plants grown in gnotobiotic systems. However this effect was reversed due to coinoculation with *Sinorhizobium meliloti* EFB1 (Villacieros et al. 2003). Different strains of antibiotic producing *Streptomyces* used to control leaf spot in alfalfa could inhibit in vitro growth of *S. meliloti* but did not significantly reduce the number of nodules. However there was an adverse affect on the growth of plant. It was postulated that the number of *S. meliloti* per nodule or their metabolic activity might have been reduced leading to diminished nitrogen fixation. It is also possible that the *Streptomyces* strains produced plant growth-inhibiting compounds (Samac et al. 2003). However, there is a report on the increase in nodulation frequency, possibly at the level of infection by *Rhizobium* sp. on inoculation of root colonizing actinomycetes *Streptomyces lydicus*. It was shown that *S. lydicus* colonizes and then sporulates within the surface layers of the nodules. Colonization leads to an increase in the average size of the nodules that form and improves the vigour of bacteroids within the nodules by enhancing nodular assimilation of iron and possibly other soil nutrients (Tokala et al. 2002). Another interesting mechanism of enhancement of growth, nodulation and nitrogen fixation of alfalfa has been reported for toxin releasing *Pseudomonas* pv. *tabaci*, a tobacco leaf pathogen. The toxin, tabtoxinine-β-lactam, inactivates selectively one form of glutamine synthetase in the nodules. Thus, normal glutamine synthetase-catalysed ammonia assimilation is significantly impaired, yet these plants assimilated about twice the amount of nitrogen (Knight and Langston-Unkefer 1988). These increases as well as concomitant decrease in glutamine synthetase activity are observed only in plants infested with pv. *tabaci*. Higher assimilation of nitrogen even in the presence of low amounts of glutamine synthetase suggests the operation of alternative routes of ammonia assimilation like through glutamate dehydrogenase and asparagine synthetase. It was further demonstrated that the changes in glutamine synthetase activity in nodules and roots of pv. *tabaci*-infested plants resulted in altered glutamate and glutamine pools; these changes may collectively influence nitrogen fixation and assimilation as well as nodulation in these infested plants. In another study the impact of biological control strain *Pseudomonas fluorescens* CHAO and its genetically modified, antibiotic over-producing derivative CHAO/pME3424 on a reconstructed population of *Sinorhizobium meliloti* bacteria was assessed in gnotobiotic systems. In sterile soil there was growth inhibition and the population of *S. meliloti* declined due to production of pyoluteorin. In plant tests, inoculation of over producing derivative did not affect nodulation, however, lack of plant growth promotion was observed. In contrast, addition of wild type strain CHAO significantly improved shoot dry weight of alfalfa plants (Nieman et al. 1997). Stimulation of plant growth by coinoculation of alfalfa with *S. meliloti* and *P. fluorescens* has also been reported previously (Liste 1993). *Pseudomonas* species isolated from the rhizosphere of green gram could inhibit the growth of several pathogenic fungi and enhanced the nodule number and growth of green gram and chickpea on coinoculation with specific rhizobia (Sindhu et al. 1999, 2002).

7.3
Interaction Between AM Fungi and *Rhizobium*

Associative effects of AMF with rhizobia have been reviewed earlier (Saxena et al. 2002) and reported for crop legumes like soybean (Zhao et al. 1997); fababeans (Ishac et al. 1994); Medicago (Azcon et al. 1991); green gram (Saxena et al. 1997) and tree legumes like *Anthyllis cystisoides* (Requena et al. 1997); *Leucaena leucocephala* (Dixon et al. 1993); *Sesbania* (Sengupta and Choudhuri 1995) and *Prosopis* (Dixon et al. 1993).

7.3.1
Influence on Nodulation and Nitrogen Fixation

Nodule number and biomass has been shown to increase significantly in several studies due to coinoculation of both microsymbionts (Saxena et al. 1997; Zhao et al. 1997). In *Acacia laeta* dual inoculation of *Rhizobium* and *Glomus mosseae* resulted in 176 and 305% increase in nodule biomass and number of nodules respectively (Badji et al. 1989). In black locust *(Robinia pseudoacacia)* mycorrhizal colonization increased nodule biomass, nodule number and N content by 78, 48 and 300% respectively (Olesniewicz and Thomas 1999). Sreenivasa et al. (1995) reported that AM fungi assist nodulation by *Bradyrhizobium japonicum* in soybean whereas *Astragalus sinicum* has been shown to be completely dependent on AM formation for nodulation (Zhao et al. 1997). However, *Glomus etunicatum* colonized soybean plants with the highest nodule numbers have been shown to have the shortest colonized root lengths (44%) while high levels of AM root colonization (76%) in *Glomus mosseae* and *Gigaspora rosea* treated plants were accompanied by low nodule counts indicating antagonism between the two symbionts (Schreiner et al. 1997). Such negative relationships between rhizobia and AM fungi have been related to competition for nutrients (Bethlenfalvay 1992) and to selective compatibilities between the microsymbionts of the legume association (Azcon et al. 1991).

7.3.2
Specific Compatibility and Yield

Like all symbiotic parameters, yield of legumes coinoculated with AM and rhizobia has been reported to increase significantly when compared to uninoculated or inoculated with either microsymbiont (Corbera and Hernandez 1997). However neutral or negative response to inoculation has also been reported (Saxena et al. 1997). Several studies have shown that there is competition for carbon between plants, mycorrhiza and bacteria in symbiotic nitrogen fixing systems (Michelsen and Sprent 1994). For example, in soybean seedlings, development of both microsymbionts is reduced in the tripartite association when compared with plants colonized by only one symbiont (Brown and Bethlenfalvay 1986, 1987). It is believed that mycorrhizal roots constitutes a stronger sink for assimilates than non-mycorrhizal roots (Smith and Gianinazzi-Pearson 1988). Such negative interaction could be due to specific compatibilities between the two microsymbionts (Saxena et al. 1997). Pacovsky (1986) also claimed that by changing *Bradyrhizobium* strain, mycorrhizal plants have shown improvement in the yield of soybean.

Selective interactions between the two endosymbionts can enhance the yield of crop plants. Combinations of *Bradyrhizobium japonicum* ICA-8001 gave best results for soybean in combination with *G. fasciculatum* (Corbera and Hernandez 1997). In a recent study, pea plants were inoculated with the AMF species *Glomus clarum* NT4 or *G. mosseae* NY6 and/or ten *Rhizobium* strains. The growth and yield response of pea to coinoculation with AMF and *Rhizobium* strains depended on the particular AMF-*Rhizobium* combination. Careful co selection of AMF species and *Rhizobium* strains enhanced pea yield and nutrition (Xavier and Germida 2003). Specific compatibilities have also been reported for other hosts like *Anthyllis cystisoides* (Requena et al. 1997); *Medicago arborea* (Valdenegro et al. 2001); kidney bean (Daniels-Hylton and Ahmad 1994) green gram (Saxena et al. 1997) and *Acacia tortilis* (Andre et al. 2003).

Plant or genotype dependent functional specificity between the microsymbionts has also been reported (Chang et al. 1992). The symbiotic parameters and yield of soybean cv. Ludous improved with a combination of *Rhizobium* 2048 and *Glomus versiforme* whereas that of cultivar zhongdous 14 improved with *Rhizobium* 61A76 and *Glomus mosseae* (Chang et al. 1992). Such plant mediated interactions could be dependent on differences in the metabolic impact of the microsymbionts on plant metabolism (Brown et al. 1988).

Specific compatibility between microsymbionts can exist both in terms of competitive ability of introduced rhizobia and percent root colonization by introduced AM fungi (Saxena et al. 1997). Thiagarajan and Ahmad (1993) reported that in the presence of AM fungus (*Glomus pallidum*), introduced strains of *Bradyrhizobium* become more competitive than native rhizobia to nodulate cowpea. Nodulation competitiveness of introduced *Bradyrhizobium* sp. (*Vigna*) strain S24 was significantly higher (60–65%) in the presence of *Glomus mosseae, G. fasiculatum* and *Scutellospora calospora* when compared to treatment with single inoculation of S24 (50%) (Saxena et al. 1997). They further reported that percentage AM colonization was higher in treatments having higher nodule occupancy of introduced strain.

7.3.3
Interaction Under Stress

The microbial-based approach, particularly use of AM fungi and *Rhizobium* for legumes has been evaluated and recommended for reclamation or for rehabilitation of desertified ecosystems (Requena et al. 1997), wastelands (Bhatia et al. 1998), saline soils (Azcon and Atrash 1997) and drought prone soils (Goicoechea et al. 1998). *Rhizobium leguminosarum* bv. *viceae* strain 102 F84 and AM inoculation in desert soils of Egypt (calcareous soils) improved growth of fababeans to a much greater extent than that can be attributed to either inoculum on a singular basis (Ishac et al. 1994). Requena et al. (1997) showed that compatible combination of AM fungi, *Rhizobium* and PGPR strain can improve plant performance in nutrient-deficient and degraded habitats. The results of a four-year trial showed that inoculation with selected rhizobia and mycorrhizal fungi improved outplanting performance, plant survival and biomass development of woody legumes in a desertified Mediterranean ecosystems (Herrera et al. 1993). Goicoechea et al. (1998) reported that symbiotic alfalfa plants are better adapted than non-symbiotic ones to cope with water deficit. It was found that symbiotic plants maintained higher polyamine (spermidine and spermine) concentrations than non-symbiotic ones under water stress.

In saline soils, the high concentration of salts is detrimental to the growth of both plant and microorganisms. Dual inoculation with AM fungi and rhizobia may help *Leucaena* and *Prosopis* species mitigate the adverse effects of NaCl on juvenile growth and development (Dixon et al. 1993; Ahmad 1996). Under salt stress, nodules formed in mycorrhizal root system were generally more abundant than the P-fertilized plants (Azcon and Atrash 1997). In stressed soils, the symbiosis with both endophytes enhances the ability of the plant to become established and cope with stress situations (nutrient deficiency, drought, trace element imbalance, soil disturbance).

7.4
Interaction of AM Fungi with PGPR

7.4.1
Mycorrhiza Development and Population Density of PGPR

AM fungi are obligate symbionts and, to date, all attempts to culture it in artificial media have failed. They survive, multiply and colonize in and around the root system. In other words, they share the common habitat that is the root surface with the plant growth promoting rhizobacteria. In the process of sharing a niche, they are bound to interact with each other and these interactions can range from beneficial to neutral to harmful. These rhizobacteria can influence the AM formation and function, and, conversely, mycorrhizas can affect the microbial population. Various spore associated bacteria including *Pseudomonas* and *Corynebacterium* have been reported to stimulate the spore germination in case of *Glomus versiforme* (Mayo et al. 1986). Cell free extracts of free living nitrogen fixing microorganisms like *Azospirillum* and *Azotobacter* significantly enhanced the spore germination of *Glomus fasciculatum*. However, non-nitrogen fixing organisms except *Pseudomonas putida* and *P. fluorescens* did not show significant increase in spore germination (Tilak et al. 1990). In contrast strains of *Pseudomonas cepacia* R85 and *P. putida* were shown to inhibit the germination of spores of *Glomus clarum* NT4 (Walley and Germida 1997). Mycorrhiza-helper bacteria are known to stimulate mycelial growth and enhance mycorrhizal formation (Gryndler et al. 2000). Soil microorganisms can produce compounds that increase root cell permeability, increase the rates of root exudation and in turn stimulate mycorrhizal fungal mycelia in the rhizosphere or facilitate root penetration by the fungus (Barea 2000). *Glomus fasciculatum* in association with *Zea mays* or *Trifolium subterraneum* reduced the viable counts of fluorescent pseudomonads (FP) but increased total bacterial numbers compared to non AM plants (Meyer and Linderman 1986), while the total viable counts of bacteria in the rhizoplane of guinea grass was reduced by *Acaulospora laevis* but increased by *G. fasciculatum* (Secilia and Bagyaraj 1987). Since microbial activity in soil is stimulated by root exudates, root colonization by AM fungi may alter bacterial growth by changing exudation patterns (Azaizeh et al. 1995). It was reported that the mycorrhizal status of soils might selectively influence persistence of bacterial inoculants as well as affect the number of other native bacteria (Andrade et al. 1998). The population density of *Pseudomonas fluorescens* 2–79RL declined by 50% and its physiological status by one order of magnitude due to root system mycorrhizal with *Glomus deserticola*. In contrast, for *Glomus intraradices*, both the population density and the physiological status of *P. fluorescens* decreased on the mycorrhizal and non-mycorrhizal side by one order of magnitude. Thus some mycorrhizal fungi can reduce both the population density and the physiological status of certain bacterial groups in the rhizosphere (Marschner and Crowley 1996).

7.4.2
Interaction of AMF and PGPR in Plant Growth Promotion

Crop productivity is usually limited by nitrogen and phosphorus availability, particularly in the tropical soils. Recent research in association between AM fungi and free living nitrogen fixers suggests that these endophytes may provide enough phosphorus and nitrogen to enhance the growth and the yield of cereals in marginal environments. Probiotic influence of associative nitrogen fixers and those of mycorrhizal fungi in major agricultural crops have been reviewed (Barea et al. 2004). In sorghum, pearlmillet and wheat coinoculation of *Azospirillum* and AMF significantly increased the growth and chlorophyll content and mycorrhizal infection of the roots (Walley and Germida 1997). However no significant effect of coinoculation was reported on nitrogen nutrition of C_3 and C_4 plants (Barea and Azcon-Aguilar 1983). Synergism of *Azotobacter* and AM fungi have also been cited in the literature (Toro et al. 1997, 1998). Dual inoculation of *A. chroococcum* and *G. fasciculatum* enhanced root infection of AM fungi, stimulated the plant growth, and increased shoot N, Ca, Mg and K in luxor tomatoes (El-Shanshoury et al. 1989). Improved growth and yield of lettuce plants in both sterile and non-sterile soils due to simultaneous inoculation with AMF and *A. chroococcum* has also been reported (Brown and Carr 1984). The synergistic host response could be mainly due to the production of phytohormones or growth regulators by these microbes rather than the small increases in N and P availability (Barea et al. 1975).

The phosphate solubilizing microorganisms (PSM) interacts well with the AMF in P deficient soils or soils amended with rock phosphate (Poi et al. 1989). The PSM can release some phosphate from otherwise sparingly soluble phosphate sources and it was postulated that AM fungal hyphae can tap these ions and translocate them to the plant. Nevertheless, Azcon-Aguilar et al. (1986) did not find that PSM improved the utilization by mycorrhizal plants of a labelled source (^{32}P–^{45}Ca-tricalcium phosphate) of insoluble phosphate added to the soil. Inoculation of Neem (*Azadirachta indica*) seedlings with *Azospirillum brasilense* and phosphate-solubilizing bacteria (PSB) in presence of AMF *Glomus intraradices* and *G. geosporum* resulted in increased mycorrhizal colonization, greater plant height, leaf area and number, root collar diameter, biomass, phosphorus, nitrogen and potassium content, and seedling quality. Inoculated seedlings also had low root/shoot ratios and low nutrient utilization efficiencies. Populations of PSB declined with seedling growth; contrarily populations of *A. brasilense* increased. *A. brasilense* and PSB populations were related to each other and influenced root colonization by AM fungi. Microbial inoculation effects were greatest when seedlings were inoculated with a combination of microbes rather than individually. This clearly indicates that these microorganisms act synergistically when inoculated simultaneously, with maximum response being when both AM fungi were coinoculated with *A. brasilense* and PSB. The results emphasize the importance of microbial inoculations for the production of robust, rapidly growing seedlings in nurseries and illustrate the advantage of inoculating soils of a low microbial population with indigenous microbes (Muthukumar et al. 2001).

Even under adverse environmental conditions or in a polluted environment, coinoculation of PGPR and AMF can be beneficial. Of the two bacterial strains isolated from lead (Pb) polluted soil, strain A (*Brevibacillus*) alone or when coinoculated with AMF enhanced plant growth of *Trifolium pratense* L., nitrogen and P accumulation, nodule formation and mycorrhizal infection. It also decreases the amount of Pb absorbed by the plants (Vivas et al. 2003). Interaction of *Rhizobium* with AMF has been discussed in an earlier section. However, there are reports on plant growth promoting activity of *Rhizobium* in the rhizosphere of non-leguminous crops (Noel et al. 1996). There is also evidence that *Rhizobium* strains were able to colonize the rhizosphere of non-legume hosts where they establish positive interactions with AM fungi and behave as PGPR (Galleguillos et al. 2000).

In fruit crops like papaya, dual inoculation of *Azospirillum* and AMF enhanced the total dry matter and leaf area than non-colonized plants (Alarcon et al. 2002). Coinoculation effects were significant even in field-established papaya and mulberry. *Bacillus coagulans* when co-inoculated with a mixture of *Glomus fasciculatum* and *G. caledonium* increase the leaf yield in ten-year-old mulberry and fruit yield in 1.5-year-old papaya (Mamatha et al. 2002).

7.5
Conclusions

The rhizosphere is the site of intense and complex microbial activity. Microbes interact with each other and contribute to soil fertility and crop productivity. In recent years emphasis on the use of two or more microorganisms has been made with the aim of maximizing beneficial plant growth responses. It is important, therefore, to identify the best strains of beneficial microbes for the planting situation, verify their compatibility and combined efficacy, both in vitro and in vivo, and employ this combination inoculum to real agricultural situation as part of the management and production practices. Compatible combinations have been tested in field, yield increases have been attained but with limited success and consistency. Inconsistency in field experimentation can be due to poor understanding of edaphic factors, composition of native microflora and even inoculum dosage. In addition the level of interaction between the beneficial microorganisms is still an unsolved mystery. The interactions can be at genetical, molecular or metabolic level. One of the most exciting discoveries in the area of symbiotic plant-microbe interactions has been the identification of common genetic determinants underlying AM and *Rhizobium* symbioses (Duc et al. 1989). Similar studies on other systems will throw light on specific interaction among microorganisms. Recently it has been reported that there is common gene expression in *Medicago truncatula* roots in response to *Pseudomonas fluorescens* colonization, mycorrhiza development and nodulation by *Sinorhizobium meliloti*. This report supports the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms. Thus research should be directed towards deciphering the intricacies of plant-microbe-microbe interaction at the genetic level.

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