Journal of Applied Botany and Food Quality 81, 95 - 109 (2007)

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Wheat x Azotobacter x VA Mycorrhiza interactions towards plant nutrition and growth – a review

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(Received July 23, 2007)

Summary

Nitrogen-fixing and phosphate-mobilizing bateria, as well as mycorrhizal fungi, can influence plant nutrition beneficially and thus be used as biofertilizers in agriculture. This paper briefly reviews the role of wheat genotypes in the interaction of wheat with soil microorganisms like phosphate solubilizing and nitrogen fixing bacteria, specifically *Azotobacter* sp., and with mycorrhizal fungi for the development of sustainable wheat crop production. The role of rhizosphere microorganisms and the mechanisms, factors affecting response of bioinoculants and the possibilities of breeding wheat genotypes responsive to these bioinoculants for sustainable wheat production in semi-arid tropics are discussed.

Introduction

Wheat (*Triticum aestivum* L.) is one of the major sources of energy, protein and fiber in human diet, staple food for nearly 35% of the world population and hence the most important cereal crop globally. Semi dwarf, high yielding wheat varieties developed in the mid sixties led to an impressive increase in wheat yields in several conventional wheat belts (RAJARAM, 1999). However, the gain in yield based on breeding (or genetic traits) has been rather low since the mid nineties. The expected global demand for wheat in the year 2020 will vary between 840 to 1,050 million tons (ROSEGRANT et al., 1995). To meet this growing demand, the global average grain yield must increase from the current 2.5 t/ha to 3.8 t/ha.

India is the second largest producer of wheat in the world after China. In India, wheat production has increased 11 times from 6.46 million tons in 1950 to 72 million tons in 2003, while the average wheat yield has increased nearly four times from 0.66 tons/ha in 1950 to 2.7 tons/ha in 2003. However, to meet the food security requirement 1.5 million tons of additional wheat per year would be required. It will require production targets of wheat to be enhanced from 95 million tons at present to 109 million tons by 2020. Such a daunting task may not be achieved easily considering the various natural and technological constraints. The conventional breeding methods provide at best only one per cent yield gain per year against the much-needed 2 per cent yield gain consistently necessary over the years.

Biofertilizers

Biofertilizers are formulated products of living cells of different types of microorganisms that have the ability to mobilize nutrients form non-usable to usable form through biological processes. These microorganisms come from the soil biosphere and hence are parts of the plant's natural environment. The microorganisms that are in use as biofertilizers in wheat broadly include the free living and associative nitrogen fixing and phosphate solubilizing rhizobacteria and the mycorrhizal fungi that are capable of mobilizing non-available nutrients from soil and transporting them to and across plant roots, e.g. phosphorus (HAYMAN and MOSSE, 1971) and zinc (SWAMINATHAN and VERMA, 1979). To these has been recently added the plant growth promoting rhizobacteria (PGPR) which stimulate plant growth and repress root diseases by a variety of mechanisms. The contribution of vesicular arbuscular mycorrhiza (VAM) and *Azotobacter* to growth and development of many plant families has long been recognized (WANI et al., 1988). Earlier studies of such types of symbioses in wheat have described considerable variability for the colonization by VAM and response to *Azotobacter*. Even when wheat is colonized, biomass and yield improvement due to symbiosis depends on the nutrient absorbing efficiency of the fungal symbiont (HETRICK et al., 1991; VEIRHEILIG and OCAMPO, 1991), fertility of the soil (YOUNG et al., 1985) and genotype of the cultivar (AZCON and OCAMPO, 1981; HETRICK et al., 1991). A wide variation in plant dependency and degree of VAM infection was found in different cultivars of wheat *(Triticum vulgare)* in response to VAM fungus *Glomus mosse* inoculation (AZCON and OCAMPO, 1981). Comprehensive reviews examined the benefits that biofertilizers including AM association can have to increase soil fertility and crop production in sustainable farming and for agroecosystems including organic farming (WU et al., 2005; GOSLING et al., 2006).

Rationale

Wheat yield *per se* is closely associated with input responsiveness. The use of higher doses of chemical fertilizers than ever before, are needed to maintain the yield levels of wheat world over. Besides, there are large areas in Asia and Africa where wheat is grown under rainfed and/or limited water and nutrient supply situations and consequently with poor yield. In these areas, soils are generally poor in mineral nutrients, organic carbon and rhizospheric activity and crops encounter intermittent moisture stress. Also in vast areas soils are either calcareous (high pH) or acidic (low pH) and hence phosphorus (P) and zinc (Zn) fixation is witnessed. Survey of literature reveals that irrespective of the levels of nitrogen fertilizer applied to wheat crop following rice, treatment with *Azotobacter* used as biofertilizer singly or in combination with VAM, leads to yield improvement of wheat. Therefore, nowadays there is a greater awareness to use microbial bioinoculants (biofertilizers) such as *Azotobacter* and mycorrhizal fungi as a component of integrated nutrient management strategies to attain higher input use efficiency (nutrients and water), to cope with increasing fertilizer costs, and their long term adverse effects on agricultural ecosystems like increased nutrient imbalances and declining productivity, etc. The beneficial effects of the use of bioinoculants include increase in plant growth and yield, better quality, improved crop uniformity and reduction in P and micronutrient fertilizer requirement, and reduced losses due to environmental stresses and diseases (ALLEN and BOOSALIS, 1983; BEHL et al., 2006). All these benefits translate into increased profits for farmers through improved production efficiencies and increased crop values. Therefore, selection of efficient wheat varieties responsive to bioinoculants and applied inorganic nutrients for high and low input conditions in different agro-ecological zones is very important to harness synergy between crop genotypes, microbial inoculants and inputs like nutrient and water.

Vesicular-Arbuscular Mycorrhizae (VAM)

The vesicular arbuscular mycorrhiza (VAM) is a mutually beneficial symbiosis of fungi which are found associated with the majority of agricultural plants. They are ubiquitous in geographic distribution occurring with plants grown in arctic, temperate and tropical regions alike. VAM occur over a broad ecological range from aquatic to desert environments. The fungi belong to the Glomeromycota with the genera *Endogone, Glomus, Entrophosphora, Gigaspora, Acaulospora, Scutellospora.* They are obligate symbionts which have not been cultured on nutrient media yet because of their obligately biotrophic life style which makes it essential for VAM fungi to infect and spread inside a living root. The symbiotic association between plant roots and mycorrhizal fungal mycelia has been termed mycorrhizae, where endomycorrhizae (here: vesicular-arbuscular mycorrhiza) colonize the root cortical cells intracellularly (SMITH, 1980; HARLEY and SMITH, 1983). VAM colonization originates from hyphae arising from soil borne propagules. Root exudates induce chemotactic growth and branching. Upon contact with the root appresoria-like structures are formed to penetrate the root surface. Intercellular growth proceeds from which hyphae invade cortical cells. Hyphae grow into cells and differentiate into tree-like dichotomously branching arbuscules. These arbuscules do not invade the cortex cell's cytoplasma membrane but rather are surrounded by the intact plant cell membrane. Between both fungal and plant plasma membrane, an extramatrical compartment allows nutrient transfer from plant to fungus (for photosynthesis products) and *vice versa* (for nutrient and water). When colonization is well established, oval structures, called vesicles, may form for which mainly storage functions for P as phospholipids have been described (BAREA, 1991). To assess the role of VAM as modifiers of soil fertility, the main quantitative estimate to be considered is the extent of external hyphae in soil associated with mycorrhizal roots (ABBOTT and ROBON, 1985). VAM have been associated with increased plant growth and with enhanced accumulation of plant nutrients, mainly P and Zn. Cu and S are mainly increased through greater soil exploration by mycorrhizal hyphae. It has also been suggested that VAM stimulate plant growth by physiological effects other than by enhancement of nutrient uptake or by reducing the severity of diseases caused by soil pathogens.

The survival and performance of VAM fungi is affected by the host plant, soil fertility, cropping practices, soil management practices, as well as biological and environmental factors. The results of field trials conducted in India reviewed (WANI and LEE, 1995) indicated that VAM inoculations increased yields significantly in around 50% of the trials and the response varied with soil type, soil fertility, and VAM cultures. Maximum root colonization and sporulation occurs in low fertility soils. However, yield increases in barley grown in soil with 40 ppm available P (Na_2HCO_3-extractable) was observed by VAM inoculation. Internal P concentration of roots rather than external P concentration in soil controls root colonization by VAM function (MENGE et al., 1978). Application of farm yard manure stimulated VAM, while in bare soil between harvesting of one crop and sowing of the next crop during the rainy season mycorrhizal spores in soil were reduced significantly. Longer periods free of cultivation reduced mycorrhizal colonization of crops, and cereals in rotation with legumes showed higher root colonization and higher numbers of VAM propagules than cereals grown in monocropping (HARINIKUMAR and BAGYARAJ, 1988). Higher temperatures generally result in better root colonization in the temperate zones. However, the reverse may be true in the tropics. Application of fungicides (like benomyl), soil solarization and prolonged water logging can significantly reduce the number of VAM propagules in soil (WANI and LEE, 1995). LUTTGER (1996) found no infection with VAM even though about 170 spores existed on one experimental field where sewage water was used for irrigation. Reasons for that might be either a high C and N content of the field, or the sensibility of VAM-spores to the pollutants in sewage water.

A key determinant for the ability of a root systems to acquire nutrients from the soil is the extent to which it is colonized by appropriate mycorrhizal fungi. The mycorrhizal condition is actually the normal status of most terrestrial plants and it greatly enhances the possibilities of nutrient uptake. The formation and activity of this symbiosis, in turn, is affected by soil fertility, as the extramatrical mycelium helps the plant to acquire nutrients from the soil (BAREA, 1991). The ecological and economic interest in VAM can simply be deduced from the fact that about four-fifths of all land plants including agronomically important crops form this type of mycorrhizae which suggests co-evolution of plants and VAM.

Inoculums of the obligate symbiotic VAM fungi have to be produced in co-culture with roots of host plants. The major constraint with VAM application in annual crops has been the production of high quality, 'clean pure' inoculum of prepared fungal species and possibly even ecotypes not contaminated with other microorganisms. Since the VAM fungi are heterokaryons with up to 5000 different genotypes within the mycelium propagated from a single spore, such ecotypes might be established only by selection during growth under the specific conditions of the plant cultivation. However, companies producing VAM in certified quality are now on the rise throughout Europe.

The other interaction partner, the plant, is more easily accessible to genetic experiments and cultivar selection. One biological approach to manage VAM symbiosis is therefore to select plant genotypes for their ability to form an efficient symbiosis with the indigenous VAM population of the field soil. It is also known that crop management, i.e, crop rotation and fertilizer management, has an impact on the amount and composition of the VAM fungal population and may, therefore, have a considerable effect on its efficiency to promote crop productivity (JOHNSON et al., 1992).

VAM infection and wheat plant growth

There is little host specificity for VAM fungi, but the competitive ability of a given species with native strains may influence the dominance of a certain endomycorrhizal fungus in a root system. Cultivars of wheat have been shown to vary in the level of colonization by VAM fungi (HETRICK et al., 1992; JOHNSON et al., 1992). Furthermore, the degree to which wheat cultivars are colonized by and benefit from VAM is a plant heritable trait which can be improved through plant breeding (JOHNSON et al., 1992). Landraces rely more on this symbiosis than modern wheat cultivars. The yield improving potential of VAM should be taken into account in the breeding of nutrient efficient wheat varieties (KAPULNIK and KUSHNIR, 1991). VAM can increase wheat plant growth and yield, improve crop uniformity and reduce fertilizer and pesticide/fungicide requirements. VEIRHEILIG and OCAMPO (1991) reported that colonization of all cultivars was independent of the amount of inoculum and the time interval of inoculation. However, it has been reported that an increase in inoculum potentially enhances effectiveness (HAAS and KRIKUN, 1985), and that the time interval of inoculation may play an important role in effectiveness of VAM infection (HEPPER, 1985). After inoculation, the dry weight of some cultivars increased, which may indicate that the increased amount of VAM inoculum increased the effectiveness of colonization. However, this effect varied depending on the cultivar and the time of re-inoculation. No relationship was found between the colonization level and the effectiveness of the symbiosis (HEPPER, 1985).

The growth temperature affects the colonization level of the wheat cultivars. Colonization was enhanced at 35 over 24°C, but temperature changes did not reduce the susceptibility of the plants even when grown at low temperature (ISHAC et al., 1986). Although a similar level of VAM colonization was observed in some cultivars grown at different temperatures, VAM increased only shoot dry weight. VEIRHEILIG and OCAMPO (1991) found no significant differences in shoot dry weight and root : shoot (R:S) ratio between inoculated and un-inoculated wheat

cultivars in earlier sampling, but after 6 weeks of growth, the inoculated cultivars showed higher shoot dry weight and lower R:S ratio than the un-inoculated cultivars. Several other authors have found no relationship between the percentage of infection and the dry weight of infected plant. Hence, measurement of succinate dehydrogenase activity has been proposed as a parameter of fungal affectivity (OCAMPO and BAREA, 1985). HETRICK et al. (1991) demonstrated that plants that rely heavily on mycorrhizal symbiosis (mycotrophic versus low mycotrophic plants) have a more plastic root morphology, i.e., they maintain a less fibrous root system in response to colonization by the mycorrhizal symbiont. In facultative mycotrophs such as wheat, however, root morphology appears to be a fixed characteristic, unchanged by mycorrhizal colonization of roots. Therefore, the observed variation in root morphologies among wheat cultivars and ancestors, and similarty of morphologies within cultivars or ancestors depending on whether or not they are mycorrhizal, suggests that the morphological differences observed are genetic characteristic of the plant themselves rather than changes induced by symbiosis. LUTTGER et al. (1993) demonstrated that genotypically determined levels of infection by the indigenous VAM fungi population did have an impact on grain yield. Cultivars with high grain yield under reduced mineral fertilization exhibited two-fold more VAM infections at the stage of tillering. The higher grain yield obtained in mycorrhizal inoculated plants is due rather to an increase in grain weight. It is suggested that the mechanism by which VAM inoculation affects grain yield is established at an early stage of plant growth rather than at the later stage of maturation.

LUTTGER (1996) reported results of extensive studies on wheat-VAM interactions in low *versus* high input genotypes under different agronomic management. Dependent upon the location, irrigation, fertilizer level and previous crop, the infection of the wheat roots varied between 0-70%. The roots of the wheat genotypes fertilized with rock phosphate showed significantly higher infection compared to the fertilizer treatment with easy soluble single super phosphate. In general, an early (crown root initiation stage) and intensive colonization of the wheat with VAM was beneficial for the grain yield in two subsequent years, particularly with reduced fertilizer application. At anthesis, the VAM infection was either positively or negatively correlated with above ground biomass or grain yield, dependent on the availability of water. In general, the influence of mycorrhizal infection increased with reduced ground water table. An impact of AMF to improved nutrient uptake was only evident at the lowest fertilizer level with rock phosphate. The increased infection rates led to significantly higher P concentrations in the above-ground biomass and an increase in Zn uptake. The symbiosis of a wheat genotype with AMF seems to be under genotypic control. Independent of the location and fertilizer level, the wheat cultivars C 306 and PBW 226 had high infection rates in both years.

Phosphate acquisition and uptake

VAM enhances plant growth by improved mineral nutrition (BAREA and AZCON-AGUILAR, 1983; HARLEY and SMITH, 1983; SREENIVASA and BAGYARAJ, 1988) which includes increased P uptake by the plant occurring in three steps:

1) mobilization and absorption of phosphate from soil by VAM hyphae,
2) translocation of phosphate along hyphae from external to internal cortical mycelia, and
3) the transfer of phosphate to cortical root cells, ready to be used by the plant (BAREA,1991).

Since under normal conditions where no fertilizers are applied, the concentration of available phosphate in soil is very low (10^{-6} M), the hyphal network of VAM make closer contacts than roots with the surface of sparingly available phosphate particles in soil and absorb it more rapidly than the roots (HETRICK et al., 1996). Also, fungal hyphae may produce organic acids like citrate and affect phosphate solubilization thus improving P nutrition of the host (CLARKE and MOSSE, 1981; SREENIVASA and BAGYARAJ, 1988; MCARTHUS and KNOWLES, 1993; WEBER et al., 1993). Although genotypic and environmental factors influence symbiosis, a prolonged, stable and compatible relation between plants and VAM is based primarily upon the transfer of P from symbiont to host that has a net P deficit (BAREA, 1991). VAM thus is important for crop production on soils with high P fixation such as calcareous soils of the semiarid regions of the tropics.

The fungus obtains photosynthates and other growth factors from the host, and in turn increases the functional root surface area through hyphal extension improving absorption of nutrients and water from soil (EDRISS et al., 1984). Mycorrhizal plants frequently show resistance to drought (NELSON and SAFIR, 1982) and environmental stresses (DEHNE, 1986). Several studies have shown that biofertilization helps expansion of the root systems, leads to better seed germination and better plant growth (BAREA et al., 2005).

TARAFDAR and MARSCHNER (1994) studied phosphatase activity of VAM fungi in rhizosphere and phyllosphere with mycorrhizal and non-mycorrhizal wheat (*Triticum aestivum*). They found that in the root compartments the activity of acid phosphatase was higher than for alkaline phosphatase, with both enzymes enhanced with VAM supplied with organic P. P application also increased the percentage of infected root length and phosphatase activity was correlated with hyphal length which was greatest within 10 mm of the root surface. Mycorrhizal inoculation increased plant dry weight, P content and total P uptake irrespective of P source and soil type. Mycorrhizal infection accounts for 24-34% of the total plant P when supplied as organic P which also stimulated mycorrhizal infection and hyphal growth. They also demonstrated the efficient use of phytate P by phosphatase of mycorrhizal hyphae.

VAM infection improves P uptake per unit root length (P influx). MANSKE et al. (1995) observed that in an field trial in Northern India, the roots of all 20 wheat lines screened were infected by the native VAM fungi in the soil. Increased fertilizer doses reduced the infection by native VAM. With reduced fertilizer application AMF infection at the stage of tillering resulted in higher grain yield. MANSKE et al. (2000) concluded that plant factors influencing P uptake efficiency are mainly associated with root characteristics. They evaluated 42 spring wheat genotypes, grown on an acid Andisol in Mexico, with and without P fertilization to identify traits associated with improved P uptake efficiency. AMF infection rate was positively correlated with P uptake in to wheat shoots (r=0.47). However, this explains only 25% of the variance of P uptake. Other traits like RLD (root length density) and phosphatatses excretion by the roots were also important. VAM-colonization and acid phosphatase activity were to a lesser degree correlated with plant P uprake efficiency.

YAO et al. (2001) evaluated three wheat (*T. aestivum*) genotypes with high (81(85)), intermediate (Fengxiao) and low (NC37) P efficiency in a pot experiment with low or adequate P supply either inoculated with the arbuscular mycorrhizal fungus *Glomus versiforme* or uninoculated. The mycorrhizal dependency of the genotypes with relatively high P efficiency was lower than that of the genotypes with lower P efficiencies. Linear correlation analysis revealed that mycorrhizal dependency was primarily controlled by P uptake efficiency. In the second pot experiment the same three genotypes were grown in low P soils. Higher hyphal length density arising from carbohydrate translocation led to more P uptake and this may account for higher mycorrhizal dependency. MANSKE and VLEK (2002) emphasized the importance of wheat nutrient uptake efficiency and suggested that genetic variation can be exploited to manipulate root characteristics such as RLD to improve nutrient use efficiency in different agro-ecological niches.

Plant growth response to VAM infection also depends on the balance between the costs and benefits of the symbiosis. The energy required

to build and maintain an extensive AMF-infected root system needs to be offset by the improved nutrient uptake of the plant, a condition that may apply in marginal soils. From the plant's side, VAM fungi are strong competitors for assimilates which is especially relevant if C availability for growth and grain filling is limited. Depending on the host plant genotypes or on P supply, 4-14% of the photosynthates are allocated to AMF-infected roots (HARRIS and PAUL, 1987). Response of plant growth to AMF usually declines as stress conditions are relaxed, e.g. sufficient nutrients are supplied. The enhanced P uptake is gained at the cost of host photosynthates utilized by AMF. However, if P becomes non-limiting and photosynthetic utilization by AMF is not curtailed, the AMF plant may become carbon limited, a situation that leads to parasitic effects of AMF (VLEK et al., 1996). NEUMANN and GEORGE (2004) conducted studies to quantify the contribution of AMF to P nutrition of the host plant when the P availability of the soil was limited by drought. To investigate the potential of AMF hyphae in taking up P from dry soil, mycorrhizal (+M) and non-mycorrhizal (-M) *Sorghum bicolor* L. plants were grown in a vertical split root system that consisted of two compartments placed one upon the other. The upper compartment was filled with well fertilized soil and the plant roots were allowed to grow into the lower compartment through a perforated bottom. The lower compartment was filled with an expanded clay substrate and nutrient solution, to supply the plants with water and all nutrients except P. The soil in upper compartments was either dried or kept moist during a period of four weeks before harvest. The total plant P content did not differ significantly between the mycorrhized and non-mycorrhized plants within the treatment with sufficient water. In contrast, the P content of mycorrhizal plants was almost doubled when the soil in the upper compartment was dried. The concentration of all elements except P in plant shoot tissue was sufficient for adequate plant growth. P concentrations in the shoots of non-mycorrhizal, water stressed plants indicated P deficiency, and these plants also had significantly lower dry matter and transpiration compared to the plants in all other treatments. The authors concluded that plant mycorrhizal colonization seems to be particularly beneficial to P uptake from dry soil.

Wheat genome and VAM infection

Variation in response to VAM is partly governed by the difference in the genetic background of the host plant and only partly by differences in the VAM genotype. Bread wheat is a hexaploid species with three different genomes (A, B, D), which can be used to study genomic effects and/or interaction in response to VAM inoculation through analytical breeding. Furthermore, modification of the VAM response during wheat evolution or domestication can be detected. The active role of the host plant and the importance of the host genotype in supporting the symbiosis and the benefits from it might be of considerable practical importance. Such information is most valuable in breeding programs, especially for developing wheat cultivars adapted to marginal soils where mycorrhiza is most effective. KAPULUIK and KUSHNIR (1991) reported that mycorrhizal dependency was higher in representatives of D genome donors. The nature of the response to VAM in hexaploid wheat was controlled by the factors of the A and B genomes which are epistatic over those located in the D genomes. The high mycorrhizal colonization and VAM dependency which was found in *T. timophivee* Var. araraticum may indicate special genomic affinity possessed by the G genome of wheat in VAM interact ion.

HETRICK et al. (1991) observed that there was no mycorrhizal dependence in *Triticum monococcum* (A genome), *Aegiops speltoids* (S (B) genome) or in AB genome ancestors *Triticum carthlicum, T. orientale, T. paleocoichicum* and *T. persicum*. Mycorrhizal dependency in D genome ancestors was more variable, as seen with *T. tauschi* var. typica which lack dependency whereas, *T. tauschi* var. meyeri and var. strangulata were dependent on mycorrhizal symbiosis. The spring wheat cultivars Chinese Spring 3008, Spelta 2603, Pavon 762980 and Norm 293025 and the winter wheat cultivars TAM 200, Wrangler, Saluda and Karl were not dependent on mycorrhizae, whereas winter wheat cultivars TAM 107 and Century were dependent. Therefore, it has been concluded that dependency in diploid ancestors with the A, S (B) and D genomes existed, but dependency was lost in tetraploid ancestors. HETRICK et al. (1991) suggested that presence of dependency in modern wheat cultivars of ABD genome is probably derived from the D genome.

It is thus evident that response to mycorrhizae is considerably influenced by the host genotype especially in a facultative mycotrophic plant such as wheat. Assuming that genetic control for mycorrhizae is oligogenic or polygenic, it is expected that genes governing host response are scattered over the A, B and D genomes of hexaploid wheat. Disomic substitution lines of chromosomes of mycorrhiza responsive C591, an old Indian wheat variety bred into the background of less responsive genotypes of Chinese Spring will be useful genetic material to elucidate chromosome specific response to mycorrhiza. Laxminarayan et al (1993) found that there are significant differences both between A, B and D genome and between seven disomic chromosomes of each genome. They found that 3A and 3D were in general responsive to *Azotobacter* while 4A and 4D showed responsiveness to mycorrhiza, and 7B to *Azospirillum* in comparative evaluation of C591 disomic substitution lines.

Azotobacter

Azotobacter chroococcum, a gram negative bacterium belonging to the family *Azotobacteraceae* of the proteobacteria is a coherent group of aerobic, free living diazotrophs able to fix atmospheric nitrogen in nitrogen free or nitrogen poor medium with organic carbon compounds as energy source. Several properties of *Azotobacter* are considered to be responsible for their beneficial effects on associated plants. These include:

• the ability to produce ammonia, vitamins and growth substances which enhance seed germination (BROWN,1975; NARULA et al., 1981; NARULA and TAURO, 1986). MERBACH and RUPPEL (1992) found that in pot experiments with soil as substrate, microbial colonization of wheat (*Triticum aestivum* L.), resulted in higher rates of $^{14}CO_2$ uptake, of ^{14}C release by roots and of ^{15}N uptake compared with plants in sterile cultures.

• the production of IAA and other auxins, gibberellins and cytokinins (MARTINEZ-TOLEDO et al., 1988) which help enhancing root growth and nutrient adsorption. SCHOLZ-SEIDEL and RUPPEL (1992) investigated the superanatant of D5/23, a free living beneficial strain isolated from the phyllosphere of winter wheat. Nonhydroxylated cytokinins ($i^6A.i^6Ade$) were detected by ELISA and two auxin compounds, 3-indole- acetic acid and 3-indole-lactic acid were detected by HPLC.

• the inhibition of phytopathogenic fungi through production of antifungal substances (SHARMA and CHAHAL, 1988; VERMA et al., 2001).

• the production of siderophores (NEILAND, 1981; PAGE, 1987; SUNEJA and LAXMINARAYANA, 1993; SUNEJA et al., 1996) which solubilizes Fe^{+3} and suppress plant pathogens through iron deprivation. *Azotobacter vinelandii* is also reported to have the ability to synthesize siderophores under Fe deficient conditions (TINDALE et al., 2000).

The numbers of *Azotobacter* in soils in various parts of the world are usually below 10^4 cells g^{-1}. A*zotobacter* develops more intensively in the root zone of plants than free in soils (DEY, 1973). These findings suggest that the root secretion of the plants may be providing the means of existence to *Azotobacter.*

Recently, BEHL et al. (2006) have reviewed role of *Azotobacter* in sustainable wheat production in semi arid tropics. Therefore, only our recent work on various aspects of *Azotobacter* and methods used by some German researchers in *Enterobacter radicincitans*, which can be used in future research of *Azotobacter* are briefly described.

MANSKE et al. (2000) found that wheat varieties showed distinct differences in the response of grain yield to *Azotobacter* inoculation. When grain yield was improved, P and N utilization efficiency were also enhanced, especially in cultivar WH542. In contrast, some cultivars improved their P and N uptake efficiency without transferring this advantage into higher grain yield (cultivars WH157 and CPN3004). In WH147 and HD2329, *Azotobacter* usually led to greater effect on improved P versus N uptake efficiency. Increase in uptake of plant nutrients (N,P,K) in wheat upon inoculation with *Azotobacter chroococcum*, both under green house and field conditions, has also been reported (KUMAR et al., 1999; NARULA et al., 2000; KUMAR et al., 2001a,b). MRKOVACKI and MILIC (2001) elaborated that bacteria of the genus *Azotobacter* synthesizes auxins, cytokinins and GA-like substances and these growth promoters are primary substances controlling the enhanced growth. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants.

In order to guarantee the high effectiveness of the inoculants and microbial fertilizers it is necessary to find the compatible partners, i.e. a particular plant genotype and a particular *Azotobacte*r strain that will form a good association. VASUDEVA et al. 2002 reported response of disomic chromosome substitution lines of wheat variety C591 to *Azotobacter chroococcum* strains Mac27 and Mac37 in a pot experiment using different soil types. They observed significant differences in viable count, plant growth parameters and nutrient uptake of different disomic lines. In general, allelels for response to *Azotobacter chroococcum* inoculation were found to be spread over all the three genomes (A, B, D). However, significant differences among different disomic lines both within a genome and over genomes were evident. Due to energetic limitations the microbiological associative nitrogen fixation from the air alone is not able to cover the nitrogen demand of the plant. It is therefore necessary to find diazotrophic strains which are able to fix atmospheric nitrogen in the presence of additional sources and to excrete nitrogen into the surrounding medium. BELA et al. (1996) developed deprepressed strains of *Azotobacter* (Mac, Ala, Msx, Mal) which can fix nitrogen in the presence of nitrogen in the soil. RUPPEL and MERBACH (1995) found that the diazotrophic strains *Azospirillum* sp and *Pantoea agglomerans* could fix atmospheric N_2 in the presence of ammonium nitrate in nutrient broth as sole nitrogen source. Further, RUPPEL and MERBACH (1997) investigated the dinitrogen fixing ability of two diazotrophic bacterial strains, *Pantoea agglomerans* and *Azospirillum* spp., in hydroponic experiments with wheat plants using $^{15}N_2$ incubation. Enrichment of ^{15}N was detected in plant growth media, and in roots and shoots of wheat plants grown 26 days in $^{15}N_2$ enriched atmosphere. Highest ^{15}N amounts were found in wheat shoots. Thus, the form of nitrogen applied and the bacterial strain inoculated affected plant growth, by nitrogen uptake and the amount of biologically fixed dinitrogen. Ammonia or nitrate supply to plants did not repress $^{15}N_2$ fixation.

The agronomic and soil health benefits are higher when the inoculated bacteria are able to survive, multiply and colonize in the rhizosphere of crop species. Large numbers of reports available in literature are based on viable counts in soil suspension samples from rhizospheric soils (BEHL et al., 2006). However, these do not furnish settlement and colonization behaviour of inoculated bacteria. This necessitate to develop methods to detect inoculated bacteria *in situ* and its settlement behaviour, on, into, or along plant roots and/or shoots, if bioinoculation technology is to be developed into a viable technology for sustainable agriculture. RUPPEL et al. (1992) investigated the ability of *Pantoea agglomerans* to colonize various regions and tissues of the wheat plant (*Triticum aestivum* L.) upon inoculation by using different inoculation methods and inoculum concentrations. In addition, ELISA and transmission electron microscopy were used to determine the ability of bacterial cells to grow and survive both on the surface and within internal tissue of the plant and the response of the plant to bacterial infection. *P. agglomerans* was found to be located in roots, stems and leaves.

Colony developments of bacterial cells were observed in leaves and stems on the surface of epidermis, in the vicinity to stomata cells, within intercellular spaces of the mesophyll and within xylem vessels. Inoculated bacterial cells were found to be able to enter host tissues, to multiply in the plant and to maintain a delicate relation between endophyte and host. Recently, NARULA et al. (2005) and VASUDEVA et al. (2007) have shown formation of paranodules (endophytic) in wheat upon inoculation with phytohormone producing *Azotobacter chroococcum* strains as well as phytohormones. KUMAR et al. (2007) studied establishment of thirteen strains of *A. chroococcum* on plant roots in relation to root exudates and development of chemotactic response in an Indian wheat variety, WH711, and two varieties of cotton (diploid and tetraploid). Analysis of root exudates revealed the presence of sugars and simple carbohydrates (glucose), amino acids (glutamate, lysine) and organic acids (citric acid, maleic acid, malonic acid). The bacterial strains showed preference for one root exudate over the others. Two strains, HT54 and IS-16, preferred wheat exudates at least two fold over cotton exudates. The other strains preferred cotton and differentiated between diploid and tetraploid species. This study revealed a high degree of adaptation between the host plant and the most abundant diazotrophs.

REMUS et al. (2000) investigated *Pantoea agglomerans* D5/23 for its colonization sites using an immunological detection method (double antibody sandwich enzyme linked immunosorbent assay, DAS-ELISA), migration within individuals of different plant species and root and shoot samples using electron microscopy. Inoculation with *P. agglomerans* led to increase in grain yield of different wheat (*Triticum aestivum*) cultivars. The same strain was also able to colonize the rhizosphere and phyllosphere of different cereals due to its ability to migrate within the plant. The authors observed that *P. agglomerans* colonized the root and plant-growth medium of wheat to a greater extent than those of rye (*Secale cereale*) and barley (*Hordeum vulgare*), whereas the colonization of shoot was higher in rye and barley compared to wheat. Thus, the host plant has also a role in determining bacterial colonization behaviour. NARULA et al. (2007) studied colonization of *A. chroococcum* strain Mac27 on wheat roots in terms of colonization sites, migration and survival of the bacteria. Also, the affectivity of inoculation of *A. chroococcum* and *P. agglomerans* D5/23 strain on wheat plant parameters under green house condition was investigated. Root tips had significant titres of inoculants as compared to the basal root parts. *A. chroococcum* colonized roots as well as soil and also migrated along roots. Both *A. chroococcum* and *P. agglomerans* were found to increase plant growth, plant dry matter, as well as N and P uptake.

Depending upon two elements of diversity, i.e. abundance and adaptability, soil microorganisms impact many soil processes and productivity. Knowledge of their interactions, roles and functions is therefore, vital to our understanding of soils and their sustainability. With the advent of recent techniques like real-time PCR it is now possible to quantify and localize specific bacterial target genes in plant tissue. A significant colonization of *Brassica oleracea* leaves and roots with *Enterobacter radicincitans* cells was measured (RUPPEL et al., 2006). The introduced bacteria proliferated on and inside the root and that they colonized the intercellular spaces of the root cortex layer.

It would interesting to know more about interaction between N availability and prokaryotic diversity particularly in a well-characterized system for long-term field experiment if biologically oriented sustainable wheat production strategies are to be developed and validated. RUPPEL et al. (2007) measured the highest prokaryotic potential functional diversity and community composition on a loamy sandy soil without N fertilization, indicating an efficient as well as versatile utilization of the substrates in this soil. Both substrate utilization richness and substrate utilization evenness, the two constituents of

the functional diversity, decreased with increasing N supply. These differences suggest a dominance of population adapted to utilizing readily available substrates. Also, prokaryotic diversity and N availability were interrelated in this sand soil system.

Azotobacter is the favoured bioinoculants promoted for cotton-wheat cropping systems in India. BHATIA (2006) investigated 76 free-living diazotrophs isolated from cotton crop soils. All these isolates were found to be Gram-negative, and morphological and further biochemical characterization also showed close resemblance of these isolates to *Azotobacter* spp. divided the isolates into two different clusters and four sub-clusters. Thus, regional specificity was observed in 16S rRNA analyses, which could be due to domestication of these isolates in different agro-ecological niches.

Different methods based on serology, homology of amplified DNA, protein and plasmid profiles have been used for strain identification. All these methods are laborious and time consuming. None of these techniques provides *in situ* detection in soil. Antibiotic or ammonium analogue resistance (LAKSHMINARAYANA et al., 2000; WILSON et al., 1994) or introduced genes that can be easily monitored on a chromogenic substrate (WILSON et al., 1994) have been proposed as a better way to identify strains.

KUMAR et al. (2006) attempted to genetically tag *Azotobacter chroococcum* strains with *lacZ* and *gfp* to study the colonization behaviour on wheat (*T. aestivum*) and cotton (*Gossypium sp.*) in soil under controlled conditions. 10^3-10^4 cfu g^{-1} soil of strain HT 57 *lac Z* were found to colonize roots of both cotton and wheat crops whereas 1.7 x 10^4 - 7.2 x 10^4 cfu g^{-1} soil of strain E12 *gfp* was colonizing wheat roots and 1.6 x 10^4 - 9.3 x 10^4 cfu g^{-1} soil of E12 *gfp* colonized cotton roots. Tagged strains were found to colonize mostly root tips in both crops.

Fate of inoculated strains in the rhizosphere

1. Crop productivity, in general, is determined by plant genotype, agronomic inputs favourable soil/rhizosphere conditions including plant microbe interactions and is positively correlated with survival, multiplication and colonization of favourable micro-organisms in the rhizosphere. Inoculation of plants with an efficient strain of a microbial inoculant might lead to fast proliferation due to root exudates or other carbon source(s) upon which bioinoculants might adapt, increase in viable counts/numbers through mutualism among the rhizosphere microorganism community, inhibition of the inoculated strain due to other microorganisms/ strains or mineralization, or through mutation generating altered efficiency or altered host preference of the inoculated strain. In order to guarantee the high effectiveness of microbial inoculants it is necessary to find the compatible partners, i.e. a particular wheat genotype and a particular *Azotobacter* strain that will form a good association.

Triple interactions

Seed inoculation with rhizobacteria may also stimulate the infection of roots by the indigenous VAM community. Synergistic effects between *Azotobacter* and *Glomus* were reported (BAGYARAJ and MENGE, 1978; ISHAC et al., 1986). BAGYARAJ and MENGE (1978) recovered larger populations of bacteria (including actinomycetes) from the rhizospheres of tomato plants inoculated with the mycorrhizal fungus *Glomus fasciculatus* and *Azotobacter chroococcum* either individually or together. Plants inoculated with both *G. fasciculatus* and *A. chroococcum* had greater numbers of bacteria and actinomycetes in the rhizosphere than plants inoculated with either *G. fasciculatus* and *A. chroococcum* alone. Inoculation of tomato with *G. fasciculatus* increased *A. chroococcum* population in the rhizosphere which was maintained at a high level for a longer time. Inoculation of tomato roots with *A. chroococcum* enhanced infection and spore production by *G. fasciculatus*. The dry weights of tomato plants inoculated with both *G. fasciculatus* and *A. chroococcum* were significantly (62%) greater than non-inoculated plants. BEHL et al. (2003) observed similar effects in wheat, and BROWN and CARR (1984) found that dual inoculation of roots of lettuce seedlings with AMF and *A. chroococcum* produced larger plants than either inoculum alone in the partially sterilized, P deficient soil. SINGH (1992) found that inoculation of N_2-fixing (*Azospirillum brasilense, A. lipoferum* and *Azotobacter chroococcum*) and P solubilizing (*Bacillus polymyxa and Pseudomonas striata*) bacteria enhanced root volume and percent VAM root colonization of *Pennisetum padicillatum* in the presence of *Glomus macrocarpum*. The number of VAM spores, after the inoculation, was also increased after inoculation with these two groups of bacteria. SINGH and KAPOOR (1998) found that root colonization by VAM fungi in the sandy soil of low fertility was low. Inoculation with AMF (*Glomus sp.* 88) improved root colonization of wheat. At maturity, grain and straw yields as well as N and P uptake improved significantly following inoculation with PO_4^{3-}-solubilizing microorganisms *Bacillus circulans* and *Cladosporium herbarum* or the VAM fungus. These increases were higher on combined inoculation of P solubilizing microbes and the VAM fungus with Mussoorie rock phosphate amendment. In general, a larger population of P solubilizing microbes was maintained in the rhizosphere of wheat in treatments with VAM fungal inoculation and rock phosphate amendment. DIEDERICHS and MANSKE (1991) observed that *Azotobacter* stimulated the mycorrhization rate in wheat roots, which, in combination with the improved total root length, resulted in an even greater enhancement of the VAM infected root length. Both effects, particularly the stimulated mycorrhization of the roots, may result in an improved P uptake, especially when plant available P in the soil is low. The benefits of the *Azotobacter*-VAM association were plant genotype dependent.

Plant growth promoting rhizobacteria (PGPR) in the rhizo-mycosphere may influence mycorrhizal function and biomass of the mycosymbiont. However, definite research in this area is still scare. All rhizobacterial associations improved mycorrhization of wheat roots. However, the degree of root colonization varied not only with different PGPR species but also with different isolates in some IAA producing PGPRs which enhanced sporulation of VAM fungi by 45%. *G. fasciculatum* and *Azotobacter chroococcum* inoculation enhanced the P concentration in shoots at tillering (MANSKE et al., 2000), although there was no effect on the shoot growth at this early stage. At the same time, mycorrhization rate in the wheat roots was higher with the dual inoculation than in the non-inoculated control. This effect was not significant with *A. chroococcum* alone. However, with time (at the stage of anthesis), the treatments with *A. chroococcum* alone or in combination with *G. fasciculatum,* improved the VAM infected root length. This effect was not only caused by an improved total root length, but also by a significantly higher VAM infection. Grain yield was improved by about 5% in the ten wheat cultivars tested.

ZAIDI and KHAN (2005) studied the interactive effect of rhizotrophic microorganisms on growth, yield, and nutrient uptake of wheat (*Triticum aestivum* L.) in a pot experiment using sterilized soil deficient in available P. Positive effects on plant vigor, nutrient uptake, and yield of wheat plants was recorded after *A. chroococcum,* phosphate solubilizing *Pseudomonas striata G. fasciculatum* inoculation. The available P status of the soil improved significantly following the triple inoculation.

WU et al. (2005) evaluated the effects of four biofertilizers containing an arbuscular mycorrhizal fungus (*G. mosseae* or *G. intraradices*) with or without N_2-fixer (*A. chroococcum*), P-solubilizer (*Bacillus megaterium*) and K-solubilizer (*B. mucilaginous*) on soil properties and the growth of *Zea mays*. The application of biofertilizer containing AMF and three species of bacteria significantly increased the growth of *Zea mays,* nutritient assimilation of the plant (total N, P and K), and soil properties. The arbuscular mycorrhizal fungi had a higher root infection

rate in the presence of bacterial inoculation. By contrast, the AMF seemed to have an inhibiting effect on the P-solubilizing bacteria. The nutrient deficiency in soil resulted in a larger population of nitrogen-fixing bacteria and higher colonization of AMF (WU et al., 2005). Thus, the findings (BAGYARAJ and MENGE, 1978; SINGH, 1992; ZAIDI and KHAN, 2005; WU et al., 2005) suggest a synergistic or additive interaction between *G. fasiculatus* and *A. chroococcum* on plants, the combined inoculation with P-solubilizers and an AMF along with rock phosphate amendment can improve crop yields in nutrient-deficient soils. Rhizotrophic microorganisms can interact positively in promoting plant growth, as well as N and P uptake of crop plants, leading to improved yields.

Root exudates

The associated heterotrophic rhizobacteria of the rhizoplane and rhizosphere depend on the carbohydrates exuded by the plant roots. BARBER and MARTIN (1976) determined that under sterile soil conditions between 5 to 10% of the net photosynthates of wheat may be released by the roots, whereas 12 to 18% is released in the non-sterile system. The latter amount of carbon release would approximate 10 to 25% of the dry matter increments of the plants. The fact, that the presence of microorganisms can cause a plant to release up to 25% (MERBACH and RUPPEL, 1992) of its photosynthate through the root indicate that we may be able to modify or manipulate associative microflora by artificial inoculation of efficient strains, thus increas-ing the carbohydrate availability to VAM since there is a close relationship between VAM and rhizosphere microflora.

In order to meet each others needs, host plant and AMF adjust through metabolic changes. Consequently, AMF induced root exudation will boost microbial communities as a whole to grow in rhizosphere with beneficial effects on plant growth. Thus, dual inoculation of efficient strains of *Azotobacter chroococcum* and *Glomus fasiculatum* in responsive wheat varieties adapted to low input stress conditions could be profitably used to maximize wheat production by harnessing favourable plant-microbe interaction.

The chemotactic responses of the plant-growth-promoting rhizobacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* to roots of vesicular-arbuscular mycorrhizal infected (*Glomus fasciculatum*) tomato plants were determined (SOOD, 2003). A significantly greater number of bacterial cells of wild strains were attracted towards vesicular-arbuscular mycorrhizal infected tomato roots compared to non-vesicular-arbuscular mycorrhizal tomato roots. Substances exuded by roots served as chemo-attractants for these bacteria and also for the VAM. *P. fluorescens* was strongly attracted towards citric and malic acids, which were predominant constituents in root exudates of tomato plants. *A. chroococcum* showed a stronger response towards sugars than amino acids, but the response was weakest towards organic acids. The effects of temperature, pH, and soil water matric potential on bacterial chemotaxis towards roots were also investigated. In general, significantly greater chemotactic responses of bacteria were observed at higher water matric potentials (0, -1, and -5 kPa), slightly acidic to neutral pH (6, 6.5 to 7), and at 20-30°C (depending on the bacterium) than in other environmental conditions. It is suggested that chemotaxis of *P. fluorescens* and *A. chroococcum* towards roots and their exudates is one of the several steps in the interaction process between bacteria and vesicular-arbuscular mycorrhizal roots.

Possible mechansims mediating wheat-VAM-A*zotobacter* interactions

The synergistic or additive interactions between VAM and *Azotobacter* could be attributed to several mechanisms which are summarized below.

1. Hormonal effects

a. Plant growth

Azotobacter is one of the microorganisms which are able to synthesize large amounts of biologically active substances. It has been reported that *Azotobacter chroococcum* produces plant growth regulators in N-free media. It is known that the beneficial effects on plant growth that result from inoculation with *Azotobacter* are caused by these growth regulators, besides its main function of N_2-fixation (BROWN, 1974). Several metabolites are contained in cell free supernatants of *Azotobacter* cultures including auxins, gibberellins and cytokinins (AZCON and BAREA, 1975; MARTINEZ-TOLEDO et al., 1988). Auxins among several other activities, control root formation and relax the cell wall, gibberellins increase leaf and root growth (PALEG and WEST, 1972) and cytokinins are involved in many basic processes of plant growth. All these activities could additionally be expected to influence the formation and function of mycorrhizae.

Two possible effects were suggested by CARR (1981) for the stimulating effect of *Azotobacter* on mycorrhiza: if absorbed into the roots, it can directly enhance of the metabolic activity of the mycorrhiza, or the increasing leaf size could enhance the potential for photosynthesizing nutrient supplies for the endophytes within the plants. TSAVKELOVA et al. (2006) reviewed the microbial producers of plant growth regulators and their practical use. Phytohormones are viewed as specific mediators in interaction between various organisms inhabiting the same ecological niche, the biological role of which is not limited to processes taking place in plants. However, specific compounds leading to such changes are yet to be identified.

b. Enhanced nutrients uptake

This effect may be related to hormone production by *Azotobacter.* These substances are known to increase the number of root hairs, lateral roots and root mass. Hence, the absorptive capacity of the root system for nutrients is expanded directly due to enhanced root growth or indirectly due to the presence of more sites created for VAM development. These effects may explain the significant increase in nutrient uptake by plants dually inoculated with *Azotobacter* and VAM compared with those singly inoculated with VAM.

2. N_2-fixation

N_2-fixation would be expected to be the principal mechanism by which *Azotobacter* interacts with AMF. The possible affinity of *Azotobacter* for VAM may be mainly based on phosphate availability. VAM enhances phosphate availability (GERDMAN, 1968) and P deficiency is known to limit N_2-fixation. AMF may supply *Azotobacter* with an adequate phosphate source in exchange for nitrogen. However, results of N_2-flxation in plants dually inoculated with *Azotobacter* and VAM did not show conclusive information.

The functional relationship between plant-VAM-rhizobacteria is complex and many questions are still open (BONFANTE, 2003). The effects of triple interactions vary due to genetic variability in each partner and their interaction for nutrient and water use and induced disease resistance, signal molecules produced by and receptors in each partner are not well understood to characterize triple interactions. Understanding of these signaling pathways and symbioses will lead to the development of mixed inocula for a given genotype of wheat and thus open new avenues for the practical use to harness the potential of bioinoculants for sustainable crop production

Genotype dependent response of bioinoculants

Genetic variability exists among wheat genotypes for acquisition and assimilation of mineral nutrients (DAMBROTH and EL-BASSAM, 1983;

MANSKE et al., 2000). EGLE et al. (1999) compared the P efficiency of three newly developed genotypes of wheat ChilWuh, BauKauz and PgoSeri with an old variety Curinda in the field at two P levels. All four genotypes responded to P application, however, the new varieties were classified as P efficient due to their significantly higher yields at both levels of P as compared to the old variety. This high level of P efficiency is mainly due to more effective P uptake. MANSKE et al. (2001) concluded, from a set of semi dwarf spring wheat (*T. aestivum*) genotypes evaluated in acid and calcareous soils without and with P fertilization, that for combining improved P uptake and P utilization efficiency in the same genotypes, simultaneous selection under both high and low P conditions is required. MANSKE et al. (2002) studied impact of Rht dwarfing genes on utilization efficiency, total P uptake and related traits in the varietal backgrounds of two tall wheat cultivars, Maringa and Nainari 60 and sets of near-isogenic lines carrying different combinations of alleles Rht-B1b, Rht-D1b Rht-B1c under conditions of adequate nutrient supply and irrigation. Dwarfing genes Rht-D1b and Rht-B1b led to improved P uptake in Maringa and P transfer in Maringa and Nainari60. The Rht-B1c genotypes showed low P uptake, thick roots and high P concentration in vegetative biomass. GILL et al. (2004) classified 30 wheat varieties by Metroglyph analysis into eight different groups on the basis of their grain yield performance and P uptake which proved useful in identifying varieties suitable for cultivation in different soil P regimes and selection of parents for recombination breeding to develop P efficient cultivars. According to this study, varieties WH711 and PBW343 with a combination of high grain yield and high P uptake will suit the high P fertility soils, whereas, Raj3765 and WH283 with high grain yield (5348 kg/ha) despite low P uptake would prove better for low P regimes as both of them recorded high index score. Inter-mating between varieties belonging to HGY-HP (PBW343 and WH711) and HGY-LP (Raj3765 and WH283) would further expand genotypic variability and thus frequency of recombinants exhibiting different grain yield and P uptake levels. This would be quite helpful to develop P efficient cultivars.

However, the effect of host genotypes on AMF infection and root characters and its interaction with host and co-inoculation with *Azotobacter* have not been adequately investigated. Although genotypic differences for AMF infection in Indian wheat genotypes under different growing conditions have been reported (MANSKE et al., 2000), information on whether such differences are heritable, as reflected in cross combinations is not available. Can this approach be effective in breeding wheat genotypes with high AMF infection and better root systems?

In order to address this question, two sets of field experiments were conducted to determine the effects of plant genotype and *Azotobacter* survival on AMF infection in some wheat crosses involving eco-geographically and genetically divergent parents grown under low mineral nutrient input conditions (for first set: SHARMA et al., 2001; BEHL et al., 2002; BEHL et al., 2003; SHARMA et al., 2006; for second experimental setup: BEHL et al., 1999; SINGH et al., 2004; SINGH et al. 2007a; SINGH et al. 2007b). Viable counts of inoculated *Azotobacter* were determined from rhizospheric soil (after BELA et al., 1986) and AMF infection in roots (after PHILLIPS and HAYMAN, 1970; JALALI and DOMSCH, 1975), in addition to measuring total root length (TENNANT, 1975).

Root and plant traits

Inoculation of AMF and AMF + *Azc* led to increase in peduncle length, flag leaf area, number of grains spike^{-1}, grain weight, biological yield and grain yield per plant (Fig. 1). Various wheat varieties and crosses showed different responses to inoculation with AMF and AMF + *Azotobacter*. Among the parents, maximum response to inoculation with AMF + *Azotobacter* was evident flag leaf area, number of grains spike^{-1}, grain yield and biological yield in WH147. In crosses, this parent contributed towards higher magnitude for these traits. Varietal response was found to be heritable. Co-inoculation of AMF with *Azotobacter* led to an increase in the viable count of *Azotobacter* in the wheat rhizosphere. AMF and *Azotobacter* complement each other and result in improved plant growth.

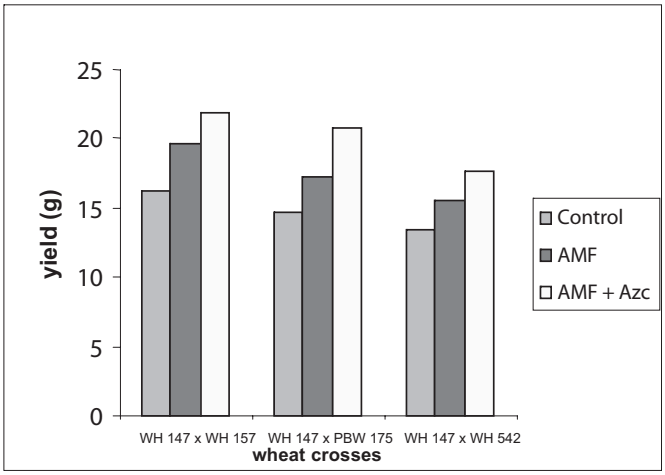

Fig. 1: Effect of bioinoculants on grain yield (g) in wheat crosses

Maximum *Azotobacter* counts were found 80 days after sowing (5.4 x 10^6) in AMF + *Azotobacter* treatments of cross WH147 x PBW175 (335 x 10^4), which may have been due to more root exudates containing amino acids, carbohydrates, organic acids and growth promoting substances (VANCURA and HARIZLIKUVA, 1972; LEINHOS and VACEK, 1994), supporting better plant-AMF-microbe interaction and nutrient mobilization efficiency of fungal (HETRICK et al., 1992) and bacterial symbionts. The bacterial population increased at faster rates in the rhizosphere of each cross when AMF was co-inoculated. It is well known that wheat roots secrete carbonaceous exudates, which could help in proliferation of AMF and *Azotobacter* (MANSKE et al., 2000). Varietal differences for root exudates thus, may be responsible for differences in viable counts of *Azotobacter* as observed.

BEHL et al. (2003) observed that wheat genotypes differed from each other for root biomass, total root length and AMF infection of roots. PBW175 followed by WH542 had maximum root biomass, root length and AMF infection in roots on the basis of two years average over all the treatments (Tab. 1). Inoculation of *Azotobacter chroococcum* along with AMF had complementary effects on AMF infection particularly in PBW 175. A comparison of the average over two years for parents and hybrids indicated that WH147 had the lowest magnitude and PBW175, on the contrary, the highest magnitude for all root traits (Tab. 1). In cross combination, PBW175 showed overdominance for root traits. Thus, the potential for higher root biomass, total root length and AMF infection of roots for PBW 175 appeared to be heritable. Although AMF infection of roots in AMF treatments was more or less similar for all crosses, inoculation of AMF + *Azotobacter* had better effects on AMF in WH147 x PBW175 and WH147 x WH157.Wheat crosses responded differently to AMF treatment as maximum increases were noted for root biomass in cross WH147 x WH157, total root length in cross WH147 x PBW175, while F_1 crosses were similar to each other for AMF infection of roots. In dual inoculation, total root length increased in each of the three crosses with a maximum being noted for WH147 x PBW175.

Micro and macro nutrient uptake

SINGH et al. (2004) showed best effects of dual inoculation with AMF (*G. manihotis*) and rhizobacteria (*A. chroococcum*) on biomass, VAM

Tab. 1: Effect of inoculation on root biomass, root length density and AMF infection in wheat

Genotype	Root biomass (g)				Root length density (m)				AM infection in roots (%)			
	C	AMF	*Azc* + AMF	Mean	C	AMF	*Azc* + AMF	Mean	C	AMF	*Azc*+ AMF	Mean
Parents												
WH147	0.74	1.09	1.20	1.00	3.60	4.07	4.52	4.06	33.1	39.3	47.5	40.0
WH157	0.63	0.95	1.15	0.90	2.24	4.12	3.75	3.37	36.3	42.5	50.4	43.0
PBW175	1.23	1.55	1.61	1.48	3.70	4.91	6.58	5.06	50.6	60.6	63.1	58.1
WH542	1.01	1.32	1.83	1.38	3.6	4.79	6.19	4.85	37.6	47.3	56.5	47.1
Crosses												
WH147xWH157	0.77	1.30	1.26	1.11	2.97	3.93	5.69	4.19	30.2	44.0	47.1	40.4
WH147x PBW175	1.05	1.08	1.37	1.16	3.66	5.04	5.66	4.78	36.6	45.2	53.3	45.0
WH147x WH542	0.83	1.02	1.02	0.95	2.95	3.56	4.63	3.71	33.4	45.7	52.8	43.9
SD	0.21	0.21	0.28	0.22	0.59	0.56	1.01	0.58	6.6	6.8	5.6	6.1

Data given represent mean values of two years.
C=control, AMF=arbuscular mycorrhizal fungi, Azc=*Azotobacter*

infection in roots and nutrient content in three spring wheat varieties and their F_1 hybrids under low pH soil (pH 3.8). Hybrid WH533 x Raj3077 exhibited higher VAM infection in roots (35.14%), P content (670ppm), Fe, Cu, Mn and Zn while WH147 x R3077 produced higher plant biomass (3.00 g plant^{-1}) and had higher N content and uptake.
Regarding micronutrients, dual inoculation of VAM and *Azotobacter* increased the Cu and Zn contents in shoots, while VAM inoculation produced better results for Fe and Mn content in the shoots (SINGH et al., 2007a). The cross WH533 x Raj3077 recorded high values for the micronutrients Fe (287 ppm) and Mn (55.0 ppm) under VAM and Zn (29.43 ppm) under dual inoculation. For Cu content, WH533 (15.6 ppm) among parents and WH147 x Raj3077 (14.80 ppm) among hybrids were best. Cross WH533 x Raj3077 exhibited heterotic effects (increased performance of F_1) for Fe contents under VAM and dual inoculation and for Cu and Mn contents under VAM influence. VAM infection in roots showed significant positive associations with nitrogen and Zn content under *Azotobacter* inoculation and with P, Fe, Cu and Mn contents were the best under VAM inoculation. Under dual inoculation, VAM infection in roots exhibited significant positive correlation with P content (r=0.65), P uptake (r=0.53), Fe (r=0.85), Cu (r=0.36) and Zn (r=0.64) content.

Gene effects

Experiments involving diverse wheat genotypes differing for their adaptation to various agronomic and ecological conditions revealed genotype dependent responses to the inoculation of *Azotobacter* and AMF individually, as well as in combination. Therefore, it was imperative to work out gene effects controlling response of inoculants for different morpho-physiological plant and root traits involved in yield building and nutrient uptake.
SHARMA et al. (2007) found that bio-inoculants enhanced the mean performance of most characters. Crop season also showed considerable effect on impact of bio-inoculants. The joint scaling test (CAVALLI, 1952) revealed adequacy of additive-dominance model of gene effects (JINKS and JONES, 1958) for root biomass, root length density, flag leaf area, tillers/plant, grain weight and grain yield in all the crosses and bio-inoculants treatments in two years. The AMF treatment brought about changes in the magnitude and significance of additive component for root biomass, plant height, flag leaf area in all the three crosses. Both additive (d) and dominance (h) components were affected with respect to grain yield in WH147xWH157 and WH147xWH542. The dominant component was important for tillers/plant, grain yield, root length in control as well as bio-inoculant treated populations of WH147xPBW175, but treatment of AMF and AMF + *Azotobacter* reduced the magnitude of h and increased the magnitude of d. Digenic interactions were prominent for grains/spike in WH147xWH157. Magnitude of digenic interactions was higher under bio-inoculation. Simple pedigree and bulk pedigree methods are suggested to capitalize on adequate additive gene effects for developing bio-inoculants responsive wheat genotypes.
Dual inoculation of efficient strains of *A. chroococcum* and *G. fasiculatum* in responsive wheat genotypes adapted to low input stress conditions might be profitably used to maximize wheat production. However, intensely AMF infected roots even at moderate nutrient deficiency are important during early plant growth when roots are too small to provide high demand for minerals for shoot growth. Selection of rapid AMF colonization in wheat may lead to improved total root length and root biomass. WH147 x PBW175 can be used for breeding pure lines in wheat for sustainable agriculture (low input genotypes responsive to biofertilizers like AMF and *Azotobacter*). Likewise, selection in cross WH147 x WH157 is expected to be fruitful to develop bio-inoculant responsive genotypes for moderate soil salinity conditions. On the other hand, cross WH147 x WH542 would yield potent recombinants for high input conditions. In any eventuality, such genotypes will be efficient to make better use of applied nutrients and thus will be suitable for sustainable wheat production under diverse agro-ecological conditions. Also, such genotypes would hold promise for organic regimes as well.
SINGH et al. (2004) evaluated the impact of bio-inoculants in low input field conditions on magnitude and direction of gene effects and mean performance of some morphological and productivity traits. The application of bioinoculants influenced genetic effects like for days to flowering, days to maturity, flag leaf area, spike length, grains/spike, 1000 grain weight and harvest index where complex genetic interactions were changed to simple additive-dominance gene effects in the cross WH147 x Raj3077. Considerable magnitude of additive and additive x additive gene effects for plant height, days to flowering, grains per spike and grain weight suggested that simple pedigree selection would be effective to develop wheat genotypes which are responsive to dual inoculation of AMF and *Azotobacter*. In this context, selection of potent recombinants in crosses involving wheat variety WH147 suitable for medium fertility and water deficient conditions holds great promise to develop bio-inoculants responsive high yielding genotypes for stress prone, low input conditions.
The impact of bio-inoculants on the magnitude and direction of gene effects was assessed for and mean performance for root length density, root biomass per plant, AMF colonization in roots and micronutrients uptake (Cu, Fe, Mn, Zn) in wheat under low input field conditions (SINGH et al., 2007a). Root length density, root biomass per plant,

AMF colonization in roots and Zn and Mn content were found maximum under dual inoculation of AMF + *Azotobacter* in all the three crosses. The joint scaling tests revealed that additive-dominance gene effects were mainly operative in governing expression of root biomass, Cu and Zn content in all the three crosses under the three treatments (i.e. control, AMF and AMF + *Azotobacter*). SINGH et al. (2007b) also analyzed the data from second set of our experiments to know the impact of bio-inoculants in low input field conditions on the magnitude and direction of gene effects and mean performance of N and P use in wheat. Bio-inoculation of AMF and AMF + *Azotobacter* exhibited positive impact on mean performance of all the wheat crosses. The mean performance of AMF was maximum in the cross WH147 x WH533 for N and P response (%), N and P use index (%) and P content (ppm), whereas, for N and P uptake it was maximum in the cross WH147 x Raj3077. The N and P response and their use index were better when combined treatment of AMF + *Azotobacter* was given in all the three crosses. Adequacy of additive-dominance model for P uptake (mg/plant) in all the three crosses under all the three treatments (i.e. control, AMF, AMF + *Azotobacter*) suggested that additive (d) and dominance (h) gene effects mainly governed inheritance of this trait. In all the cases, digenic interactions were present where duplicate type of epistasis prevailed except in P content for the control in the cross WH147 x WH533, where complementary type of interaction was present. From both analyses, it appears that Pedigree selection in crosses WH147 x WH533 and WH147 x Raj3077 can be effective for breeding pure lines in wheat combining high yield and nutrient use efficiency (low input genotypes responsive to biofertilizers like AMF and *Azotobacter*)for sustainable agriculture).

Candidate genotypes for breeding of improved associative cultivars

Tall wheat varieties carry the rht gene. Such varieties produce low endogenous hormone. Supply of hormone in any form eventually leads to increase in root and shoot lengths and consequently root and shoot biomasses (GALE and MARSHALL, 1975). PBW 175 is a tall type genotype having an efficient root system (SANGWAN et al., 1999) tailored to extract more water from deeper soil profiles under stress conditions. In one of our earlier studies, AMF infection was observed to be higher under dryness and nutrient stress conditions (MANSKE et al., 1995) in wheat genotypes tolerant to abiotic stresses (MANSKE et al., 1999). In general, the capability of rhizobacteria to produce plant growth regulating substances of the auxin type may contribute to their stimulating effects on plant growth (SATTAR and GAUR, 1987; LEINHOS and VACEK, 1994). The phytohormone indole acetic acid (IAA) affects root elongation and lateral root formation (HIRSCH et al., 1997). Plant root systems are complex and are comprised of several types of structural and functional characteristic (RENEGAL, 1997). Efficient water and mineral uptake is closely related to roots and their growth (KAPULNIK et al., 1985). The stimulation of root biomass and total root length for plants grown in dual inoculation, as observed in our studies, may be the result of cumulative effects of favorable plant-AMF-microbe interactions. Total root length (m) was maximum in WH147 x PBW175 control treatments, and increased in each cross with AMF and AMF + *Azotobacter* treatments, being lowest in WH147 x WH542. This could be possible as plants may have had fibrous and plastic roots (HETRICK et al., 1991).

The bacterial population increased at faster rates in the rhizosphere of each cross when AMF was co-inoculated. It is well known fact that wheat roots secrete carbonaceous exudates, which could help in proliferation of AMF and *Azotobacter* (MANSKE et al., 1999). *Azotobacter* excretes phytohormones, which improves growth of plant roots, and AMF may solubilize P from surrounding areas and make it available to roots. Dual inoculation of efficient strains of *Azotobacter chroococcum* and *Glomus fasiculatum* in responsive wheat genotypes adapted to low input stress conditions would be profitably used to maximize wheat production. However, intensely AMF infected roots even at moderate nutrient deficiency are important during early plant growth when roots are too small to provide the high demand for minerals for shoot growth. Selection of rapid AMF colonization in wheat may lead to improved total root length and root biomass. WH147 x PBW175 can be used for breeding pure lines in wheat for sustainable agriculture (low input genotypes responsive to biofertilizers like AMF and *Azotobacter*).

Breeding strategies

Mycorrhizal dependence is a genetic trait that is strong in some wheat genotypes and absent in other (HETRICK et al., 1992). Mycorrhizal dependence is an index of plant response at particular P level (PLENCHETTE et al., 1983). Only relative relationship between cultivars can be inferred from this index. The P level in the soil was relatively low; therefore, both the degree of growth and the number of cultivars responding to mycorrhizae would decline with increasing P level in the soil. The significant correlation between root fibrousness and mycorrhizal dependence suggests that the use of a morphological trait such as root architecture is probably a more reliable predictor of plant benefit from the symbiosis than the colonization by the fungal symbiont. Using low fertility soil and fungal symbiont infection of wheat, HETRIC et al. (1992) suggested that old hexaploid wheat, landraces and older cultivars are more consistent and highly responsive to the symbiosis than modern wheat cultivars. The germplasm selection under fertilized condition could have reduced the frequency of genes for mycorrhizal dependence in wheat as under high fertility conditions VAM infection is less and Wheat genes responsive to VAM may not be expressed.

Symbiosis creates unique problems because the phenotype is the product of an intimate association between different organisms. As a result, the development of the symbiotic phenotype can be influenced by genes which affect each organism's independent existence as well as those which determine their joint association. Such factors have the potential to produce levels of complexity between genotypes and environments that are reflected in the phenotype which are not easily unraveled and thus not easily stabilized. It is, perhaps, important to emphasize at this point that the agronomic objective is the phenotype rather than the genotype. Inferences about genotype are made from phenotype and assessment of the strength and stability of this relationship is of central importance to the speed and precision with which genetic patterns can be altered in populations. Thus, the first tasks in any breeding program are to identity and quantify phenotypic variations for relevant traits. Often environment and genotype x environment interaction influence phenotypic expression and create bias to unknown extent. Under such situation selection is jeopardized. Such a situation warrants use of DNA markers which are ubiquitous and independent of environment. However, such DNA markers with universal application are yet to be identified.

If the gene effects for VAM-wheat symbiosis are additive or additive x additive simple selection of wheat lines will be effective. However, non-additive gene effects are the basis for poor heritability, intense selection and specific combining ability in such cases are important to breed for VAM-Azotobacter responsive wheat genotypes. However, the available literature points to the possibilities that wheat response to bio-inoculants being heritable and genotype dependent, targeted and efficient breeding of wheat genotypes for biologically oriented land use system is possible using a combination of conventional and molecular approaches.

Outlook

Soil is a habitat for a vast, complex and interactive community of soil organisms whose activities largely determine the chemical and physical

properties of the soil. Soil micro flora plays an important role in the maintenance of soil fertility because of their ability to carry out biochemical transformations and also due to their importance as a source and sink for mineral nutrients. Trophic interactions between plants, soil microflora control patterns and rates of organic matter decomposition, nutrient cycling, mobilization, and nutrient uptake by plants. Soil structure and porosity are much influenced by the activities of soil organisms, especially moisture, temperature, and aeration. In turn, soil structure and porosity determine community structure and regulate soil fertility. Microorganisms also influence the crop productivity through control of insect pests and diseases in sustainable development of agriculture (LYNCH and BRAGG, 1985; BAREA et al., 2005).

Under the present day energy crisis there is an urgent need to improve the potential benefits of bioinoculants like *Azotobacter and* VAM for sustainable and enhanced wheat production for food security. PGPR have the potential to improve plant health and productivity and they are particularly suitable for low input sustainable agriculture applications, such as biocontrol.

Inoculants bacteria are often applied in seed coatings. After sowing, the inoculants bacteria must be able to establish themselves in the rhizosphere at population densities sufficient to produce a beneficial effect. Therefore, efficient inoculants bacteria should survive in the rhizosphere, make use of nutrients exuded by the plant root, proliferate, be able to efficiently colonize the entire root system and be able to compete with indigenous microorganisms. Inadequate bio-control in the field experiments has often been correlated to poor root colonization. Identification of the genes and traits involved in the processes of inoculation and root colonization will give a more detailed insight into plant-bacterial interactions and lead to more efficient application of inoculant strains. The survival and competitive ability and performance of the improved bioinoculant strains must be improved for different agro-ecological niches.

Plant species can define the bacterial communities present in the rhizosphere via the selection of genetically diverse populations or by favouring specific dominant groups. However, despite the acceptance that plant extracellular signals influence microbial populations in the rhizosphere, the most microorganisms residing there, little is known about the genes involved and roles they may play in plant-microbe interactions. Traditional molecular techniques, such as transposon mutagenesis, provide researchers with important information about the production and regulation of biocontrol determinants. Modern techniques now allow molecular studies of PGPR not just in isolation but as a part of the complex interacting communities that occur in rhizosphere (FRANKS et al., 2006). Use of PCR based molecular markers, as in case of *Enterobacter radicincitans* by some German researchers, should be made to unravel facts involved in genotype-microbe interaction in specific agro-ecological conditions to harness favourable interactions for better understanding and application. Yet our understanding of the links between microbial diversity and soil/rhizosphere functions remains poor, we have still to discover an easy and comprehensive methods for measuring functional microbial diversity among individual community members. In addition, current measurements of microbial function determine only the overall rate of an entire metabolic process and are still unable to identify directly the microbial species involved.

The characterization of novel genes involved in plant-microbe interaction is vital for understanding the response of rhizospheric bacterial populations to plant signals at a molecular level. By incorporating techniques such as *in vivo* expression technology with recent advances in transcriptome profiling, researchers can search for genes induced or repressed by plant signals. *Psuedomonas fluorescens* genes that are specifically expressed in the rhizosphere (rhi genes) have been identified using IVET (RAINY, 1999). More than 20 genes have been identified, of which 14 showed significant homology to genes that are involved in nutrient acquisition, stress response or secretion (BLOEMBERG and LUGTENBERG, 2001). Our understanding of the rhizospheric function of these genes can be furthered by application of proteomics, metabolomics and metagenomics, while marker gene technology can provide spatial and temporal information on specific gene expression. To elucidate the role and function of PGPR in the rhizosphere, a number of complementary techniques may therefore, be used to generate information and further overall bacteria-plant interactions.

Appropriate management practices and compatible wheat genotypes need to be provided to ensure maximum contribution from bioinoculants. Host controlled factors play an important role in regulating responses of bio-inoculants but not have received due share of attention by researchers. In coming years, transgenics will occupy larger areas under agriculture world over. Targeted genetic traits for improved plant nutrition include greater plant tolerance to low Fe available in alkaline soils, enhanced acquisition of soil inorganic and organic P and increased assimilation of soil N (MOTAVALLI et al., 2004). Possible changes in soil microbial activity due to differences in the amount and composition of root exudates, changes in microbial functions and alterations in microbial populations resulting from gene transfer from the transgenic crops and the effects management practices for transgenic crops, respectively would warrant evaluations of the impact of the diverse transgenic crops to an improved understanding of soil biological functions and processes.

Genotypic variation exists for VAM infection and *Azotobacter* response and the expression of variability is better under low input condition. Hence, it is possible to select and breed wheat genotypes under low input conditions. Many plant genes might be involved (quantitative trait loci, QTLs) and can be identified for their functions in host response to bio-inoculants. Pyramiding of such QTLs, mostly available in traditional varieties, local land races, should be done in the background of agronomically superior genotypes for high and low input systems. Therefore, more efforts should be done in this direction which will certainly prove effective in the advancement of wheat improvement programme for biologically oriented land use system. Transcriptomic studies related to plant-fungus combinations in different natural field conditions make important contributions to understand the ecological context of the symbiosis (FRANKEN, 2006). Besides roots, it would be imperative to study the photosynthetic part of the plant as well as the developing fruits in any transcriptome analysis as has been performed in one case for leaves of tomato plants (TAYLOR and HARRIER, 2003).

BAREA et al. (2005) described microbial cooperation in the rhizosphere. They highlighted that soil microbial populations are immersed in a frame-work of interactions known to affect plant fitness and soil quality. They are involved in fundamental activities that ensure the stability and productivity of both agricultural systems and natural ecosystems. Strategic and applied research has demonstrated that certain co-operative microbial activities can be exploited, as a low-input biotechnology to help sustainable, environmentally-friendly, agro-technological practices.

Plant growth stimulating rhizobacteria can be propagated *in vitro* in huge quantities as pure cultures in biofermenters at low cost. Diazotrophs, particularly *Azotobacter chroococcum* are increasingly being used as bioinoculants for wheat and other crops to increase crop productivity (NARULA et al., 2005) in India and elsewhere. In India, *Azotobacter* is being marketed with success over past two decades and intensive research and development efforts are being made in public and private sector institutions. However, there is still need to optimise the efficiency of these products. The discovery of many traits and genes that are involved in the beneficial effects of PGPRs has resulted in a better understanding of the performance bio-inoculants in the field, and provides the opportunity to enhance the beneficial effects of PGPR strains by genetic modification (BLOEMBERG and LUGTENBERG, 2001). Some strong correlations between the presence

of an organism with microbial processes or soil structure and function would be helpful in making wise choices prior to making large investments in such sequencing projects (KENT and TRIPLETT, 2002). As a result, linkage of specific organisms to ecosystem function over time and space is fundamental work that must go forward. Genetic tagging of *Azotobacter chroococcum* with lac Z and gfp markers as reported by KUMAR et al. (2007) might be useful in to study colonization behaviour and ecological studies besides other molecular methods.

Mutualism among *Azotobacter strains* and VAM species and wheat host genotype must be studied both *in vitro* and *in vivo* so as to find best combination for different environmental conditions. Research should be intensified to establish diversity in *Azotobacter* spp. and VAM from different wheat growing localities, an effective associative/ endophyte system in wheat by identifying effective bio-inoculants strains and plant genotypes so as to identify ideal plant genotype-microbial inoculants partners.

In order to harness synergy among wheat, VA mycorrhiza and *Azotobacter*, following aspects deserve attention for future work:

(i) identify biological markers for interaction between *Azotobacter* and AM fungi and /or PSB to select the most compatible combinations for wheat plant inoculation,

(ii) develop *Azotobacter* strains that can provide more nitrogen to plants/VAM through nitrogen fixation or excretion of ammonia without compromising survival and colonization in rhizosphere under diverse ecological conditions,

(iii) identify physiological features of bacterium that have role in microbe-microbe-plant association,

(iv) study role and regulation(gene expression) of phytohormones like gibberellins, kinetins and cytokinins and particularly IAA pathway in wheat-VAM-Azotobacter interaction,

(v) study role of root exudation and exudates in triggering and silencing bacterial genes in relation to their impact on processes like chemotaxis, survival and colonization determining plant bacterial-fungal interactions, and

(vi) use of microarrays and proteomics to elucidate cross communication among wheat roots-VAM and *Azotobacter* in the rhizosphere.

Likewise, the identification of genes involved in the yield and grain quality in response to inoculation with mycorrhiza and and *Azotobacter* as well as use of such gene as functional markers in wheat breeding or as elements of constructs for transgenic plants with improved traits may be quite useful.

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