

Exploration of Microbial Wealth for Sustainable Horticultural Production

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Abstract

Sustainable production of horticultural crops in the tropics is often severely constrained by the fragility of soils, being prone to several forms of degradation. Microbes being an integral component of any soil eco-system provide life to the soil. They render soil richness in terms of making available slow-release nutrients, continuous breaking down of complex macro-molecules and natural products into simpler one to enrich beneficial substances, maintaining good physicochemical properties of the soil and most essentially providing support to the plants in terms of growth enhancement. This review treats the role of microorganisms in improving nutrition of major (N, P and K) as well as micronutrients in horticultural crops, promoting plant growth and yield. It focuses on the mechanisms of various groups of microorganisms involved in harnessing atmospheric N₂, mobilizing insoluble source of phosphorus, potassium and micro-nutrients and promoting plant growth through improved nutrition and growth hormone production. Further improvement in the efficiency of phosphate mobilizing micro-organisms through genetic engineering has also been discussed. Thus microbial association enable a better use of sparingly soluble nutrients either inherited from soil or applied through anthropogenic sources, thereby increasing the efficiency of added fertilizers.

1. Introduction

Ancient agriculture started with shifting cultivation, which restored soil fertility. This system was later replaced by bush fallow system and organic farming, which continued till 1960s in India. During the past four decades we have witnessed the doubling of the human population and a concurrent doubling of food production. Increase in crop production has been made possible through the use of commercial man-made fertilizer and pesticides. The tremendous increase of N and P fertilization, in addition to the introduction of highly productive and intensive cultivation system has allowed these developments to occur at relatively low costs. However, due to unintended introduction of degrading process like extensive use of agro-chemicals and irrigation mediated salinization, the intrinsic capacity of the natural resource (soil) has diminished in developing as well as developed countries. Indiscriminate addition of agro-chemicals and their inefficient use by crop plants as well have led to unsustainability of a farming enterprise and also created environmental problems such as deterioration of soil quality, surface water, and ground-water as well as air pollution, reduced biodiversity and suppressed eco-system function. These have led to the development of “sustainable

agriculture” concept in mid 80s. It envisages a system approach and places primary emphasis on maximizing not only the yield but also agro-eco-system stability. Thus, it represents an integration of traditional techniques with modern advances, which are recognized as appropriate. Further, high fertilizer prices brought about by the energy crisis, cost of inputs and transportation, import restrictions and inconveniences, the need for conservation of foreign exchange necessitate serious attention to be given to a technology in agriculture/horticulture having both chemical and bio-components for proper management of soil nutrients. Thus, the rational supplementation of agro-chemical with beneficial microbes will be a healthy and promising approach for making the production system more sustainable, eco-friendly and profitable. It has been realized that microorganisms are a vital component of sustainable agro-production system (Venkateshwarlu et al., 2008). They have intimate relation with soil and are the first colonizer of our planet. They have been assisting in soil formation process and augmenting soil fertility through various activities and even all nutrient transformations are mediated by soil microbes. Some of them have the ability to harness atmospheric nitrogen and make it available to crops either directly or indirectly. Even, solubilization of phosphorus is also accomplished by many

heterotrophic soil microbes. Besides major nutrients, available micronutrient status of soil is also directly or indirectly regulated by microorganisms. To harness maximum benefit from these microbes, the need of artificial inoculation of efficient microbes was felt and accordingly bio-fertilizer technology was invoked by using these microbes as source of inoculum.

2. Nitrogen Nutrition

Atmospheric nitrogen (N) composes approximately 80% of the air we breathe. Although abundant and ubiquitous in the air, N is the most limiting nutrient to plant growth, because the atmospheric N_2 is not available for plant uptake. Some bacteria are capable of N_2 fixation from the atmospheric N pool. These bacteria form various associations with plants: many free-living N_2 -fixing bacteria occur in soil, some have adapted to form symbiosis; others have intimate endophytic association with plants, and others live in close association in the plant root zone (rhizosphere) without forming intimate endophytic symbiosis. The amount of nitrogen fixed by these different systems is considerable, although variation resulting from environmental conditions or different plant microbes combination is vast. The close proximity of these microorganisms to their host plant allows efficient plant use of fixed nitrogen and minimizes volatilization, leaching and denitrification losses.

Azotobacter is one of the important members of the free-living diazotrophic club due to its broad spectrum utility for different crops. Its colonies appear flat, soft, milky and mucoid. The cells are polymorphic and young ones have peritrichous flagella. *Azotobacter chroococcum* is the most prevalent species found but other species reported include *A. agilis*, *A. vinelandi*, *A. beijerinckii*, *A. insignis*, *A. macrocytogenes* and *A. paspali*. As it is sensitive bacterium, its establishment in soil is very poor. But the rhizosphere of the crop plants is found to be more congenial than non-rhizosphere soils. In rhizosphere root exudates, amino acids, sugars, vitamins, organic acids together with the decaying portion of root system serve as an energy source for its survival and growth. Furthermore, its proliferation in rhizosphere is influenced by the ecological and agro-climatic factors like fertility levels and soil type, moisture, temperature, pH, organic carbon content of soil, plant type, nature of plant exudates and interaction with other microbes. It was observed that *Azotobacter* population in the peach rhizosphere increased with fertilizer application upto 180 days and then declined (Godara et al., 1995). Vesicular arbuscular mycorrhizal (VAM) fungi and cellulolytic microbes encourage *Azotobacter* population (Godara et al., 1995) but *Cephalosporium* sp. may inhibit its growth (Jha et al., 1999). The potential of *Azotobacter chroococcum* as nitrogen fixer in fruit plant have been exploited. *Azotobacter chroococcum* inoculation (20 l ha^{-1}) in banana stimulated all phenological

variables (plant height, number of leaves and shoots and pseudo-stem diameter). Bacterial inoculation improved fruit development and increased the fruit: rachis ratio particularly when nitrogenous fertilizer was applied between 80% and 100% of recommended dose of fertilizer (RDF). So the bacterial inoculation could compensate for 20% of the N-fertilizer without changing the yield obtained from 100% N-fertilizer application (Dibut-Alvarez et al., 1996). Similarly, inoculation of *Azotobacter chroococcum* in 'Anna' apple along with farm yard manure and suboptimal dose of nitrogenous fertilizer increased leaf nitrogen content to a considerable extent at three growth stages and also raised fruit nitrate content significantly (EL-Boray et al., 2006). Poor establishment of *Azotobacter* in rhizosphere makes them poor competitors and that leads to erratic response. Thus, immediate need is to select or breed a strain with better efficiency and competitive ability.

The group of microorganisms, which fixes atmospheric N_2 through formation of loose association with plant root, is known as associative symbiotic N_2 -fixer. *Azospirillum* are important member of associative N_2 -fixer and are found widely distributed in loose association with roots of most of the agriculturally/horticulturally important crop plants. They have no preferences for crop plants or weeds, or for annual or perennial plants, and can be successfully applied to plants that have no previous history of *Azospirillum* in their roots. It appears that *Azospirillum* is a general root colonizer and not a plant specific bacterium. Earlier, Bashan and Holguin (1997), and Bashan et al. (2004) have provided detailed information with regard to plant species and association of *Azospirillum*. Soil inoculation with pure cell suspensions of *Azospirillum brasilense* markedly increased plant dry weight and nitrogen uptake in pomegranate, ber (*Ziziphus mauritiana*), mulberry and other fruit crops (Rao and Dass, 1989; Das et al., 1994; Hazarika and Ansari, 2007). Application of the bacterium *Azospirillum brasilense* (3 g plant^{-1} as a root dip at transplanting) to sweet orange cv. Mosambi plants substituted for at least $\frac{1}{4}$ of the nitrogenous fertilizer requirement (Singh and Sharma, 1993). Tiwary et al. (1999) also reported that *Azospirillum* inoculation in banana along with 100% recommended N-fertilizer increased leaf nitrogen and chlorophyll content to a considerable extent. Their contribution to yield of various vegetable crops like onion, brinjal and potato, etc. has also been demonstrated to be equivalent to that of $15\text{-}20 \text{ kg N ha}^{-1}$. However, magnitude of response varied with location, season, bacterial strain and crop variety, etc. (Thilakavathy and Ramaswamy, 1999; Mahendran and Kumar, 1998; Nanthakumar and Veeraragavathatham, 1999). In fact, N_2 -fixation was naturally the first major mechanism of action suggested for the enhancement of plant growth using *Azospirillum*. Incorporation of atmospheric nitrogen into the host plant by *Azospirillum* is evaluated mainly by the acetylene

reduction assay (Van BerKum and Bohlool, 1980). Evidences are there that N_2 -fixation contributes to the nitrogen balance of plants is based on the common observation of an increase in the nitrogenase activity within inoculated roots (Berg et al., 1980; Cohen et al., 1980; Kapulnik et al., 1981; Hess, 1982; Hegazi et al., 1983; Okon et al., 1983; Yahalom et al., 1984). This well documented enzymatic activity is of sufficient magnitude to account for the increase in total nitrogen yield of inoculated plants when the entire fixed N is incorporated into the plants (Sarig et al., 1984; Mertens and Hess, 1984). Further studies pinpointed the positive bacterial effects on plants influencing morphological and physiological processes in the inoculated roots that could lead to an enhancement of water and mineral uptake (Okon and Kapulnik, 1986). However, it was agreed that the beneficial *Azospirillum* effects on plants was dependent on good root colonization. Hence, root colonization is important as the first step not only in infection by soil-borne pathogens but also in beneficial associations with microorganisms. The *Azospirillum*-root interaction is a two step process comprised of adsorption, mediated by bacterial proteins, and anchoring involving bacterial polysaccharides (Michiels et al., 1991). To attach and colonize plant root surfaces, *Azospirillum* spp. must first rely in a process that depends on active motility and chemotaxis toward root exudates. Attempt was also made to study the distribution of *Azospirillum* in the root with different techniques. Using the *gfp*-protein to tag bacteria, Liu et al. (2003) confirmed previous findings about colonizing patterns. In general, the bacteria are established mainly on the root surface but some strains of *A. lipoferum* and *A. brasilense* are capable of colonizing the root interior in the apoplast and intercellular spaces. This ability could mean a lower vulnerability to harsh conditions imposed by the soil and/or the environment, which in turn could imply a more efficient promotion of plant growth (Sturz and Nowak, 2000). However, rhizobacteria established inside roots in intimate association with plants are considered endophytes. These microorganisms live outside the symplast and do not produce nodules, but can produce signal compounds that stimulate plant growth, enhance plant disease resistance and improve mobilization of soil nutrients. Arbuscular mycorrhizae (AM) have also been reported to enhance N nutrition of inoculated plant. In acid soils, AM fungi may be important for the uptake of ammonium (NH_4^+), which is less mobile than nitrate (NO_3^-) and where diffusion may limit its uptake rate. Although nitrate is much more mobile than ammonium (uptake is regulated through mass flow), AM fungi may be important in nitrate uptake in Mediterranean and semi-desert eco-systems. Because of their small size, AM fungal hyphae are better able than plant roots to penetrate decomposing organic material and therefore better competitors for recently mineralized N (Hodge, 2003). The enhancement of nitrogen uptake by pomegranate through use

of AM fungi was evident in several studies (Aseri et al, 2008; Rupnawar and Navale, 2000ab) demonstrating its usefulness in promoting N nutrition in plants. Moreover, vesicular arbuscular mycorrhizas (VAM) fungi also influence nitrogen metabolism within the plant through enhancing enzyme activity involved in the process. It has been observed that mycorrhizal inoculation in jhar-beri (*Ziziphus nummularia*) increased the activities of glutamine synthetase, nitrate reductase and glutamine dehydrogenase thereby increased the concentration of soluble protein in leaves and roots (Mathur and Vyas, 1995).

3. Phosphorus Nutrition

Phosphorus is an important element and plays a key role in the nutrition of plant as it promotes development of deeper roots (Gaur, 1990). Being a constituent of ATP, it is involved in diverse process such as cell division, energy transduction through photosynthesis and biological oxidations and nutrient uptake (Awasthi et al., 2011). There are two components of phosphorus (P) in soil, organic and inorganic phosphate. And a large proportion of it is present in insoluble forms and therefore, not available for plant nutrition. Inorganic P occurs in soil, mostly in insoluble minerals complexes, some of them appearing after the application of chemical fertilizers. However, these precipitated forms can not be absorbed by plants. Organic matter on the other hand, is an important reservoir of immobilized P that accounts for 20-80% of soil P (Richardson, 1994). Phosphorus can be released from organic compounds in soil by three groups of enzymes: non-specific phosphatase, which performs dephosphorylation of phosphor-ester or phosphor-anhydride bonds in organic matter; phytases, which specifically cause P release from phytic acid; and phosphonates and C-P lyases enzymes that perform C-P cleavage in organophosphates. The main activity apparently corresponds to the work of acid phosphatases and phytases because of the predominant presence of their substrates in soils. Availability of organic phosphate compounds for plant nutrition could be a limitation in some soils resulting from precipitation with soil particle ions. Therefore, the capability of enzymes to perform the desired function in the rhizosphere is a crucial aspect for their effectiveness in plant nutrition. Several acid phosphatase gene from gram-negative bacteria have been isolated and characterized (Rossolini et al., 1998). The *acpA* gene isolated from *Francisella tularensis* expresses an acid phosphatase with optimum action at pH 6.0 with a wide range of substrate specificity (Reilly et al., 1996). Also genes encoding non-specific acid phosphatase class A (*PhoC*) and class B (*NapA*) isolated from *Morganella morganii* are very promising since the biophysical and functional properties of the coded enzymes were extensively studied (Thaller et al., 1994, 1995). Heterologous expression of these genes in agriculturally important bacterial strains would be the next step in programs of improving organic

phosphate mineralization in plant growth promoting bacteria (PGPB). The *napA* phosphatase gene from the soil bacterium *Morganella morganii* was transferred to *Burkholderia cepacia* IS-16, a strain used as bio-fertilizer, using the broad host range vector pRK293 (Fraga et al., 2001). However, an increase in extracellular phosphatase activity of the recombinant strain was reported. Insertion of transferred genes into the bacterial chromosome is advantageous for stability and ecological safety. A plasmid for the stable chromosomal insertion of the *phoC* phosphatase gene from *Morganella morganii* was constructed based on the delivery system developed by de Lorenzo et al. (1990). Further, this plasmid was transferred to *Azospirillum* and preliminary results indicated increased phosphatase activity of the strain.

Similarly, thermally stable phytase gene (*phy*) from *Bacillus* sp. DS 11 (Kim et al., 1998) and from *Bacillus subtilis* VTT E-68013 (Kerovuo et al., 1998) has been cloned. Acid phosphatase/phytase gene from *Escherichia coli* (*appA* and *appA2* genes) have also been isolated and characterized (Rodriguez et al., 1999; Golovan et al., 2000). The bi-functionality of these enzymes makes them attractive for solubilization of organic P in soil. However, neutral phytase have also great potential for genetic improvement of PGPB.

Even many fungi, bacteria, actinomycetes and cyanobacteria are potential solubilizers of bound phosphorus in soil (Singh and Kapoor, 1992; Gupta et al., 1998; Whipps, 2001; Hu, 2005; Zaidi and Khan, 2006; Chakraborty et al., 2006; Siddiqui, 2006; Awasthi et al., 2011). Phosphate solubilizing microorganisms are found in all soils but their number greatly varies with soil climate and its history (Gupta et al., 1986). Though, they are more common in the rhizosphere soil of different crops than that in non-rhizosphere soil. Even, few of them were also reported from the phyllosphere of crop plants. Interestingly, their presence was also noted in the soils of rock phosphate deposit area and marine environment. The most efficient phosphate dissolving bacterial isolates belong to the genera *Bacillus* and *Pseudomonas*, though species of *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Sarcina* and *Achromobacter* are found to be active in solubilizing the insoluble phosphate. Among fungi, the most efficient phosphate solubilizers belong to the genera *Aspergillus* and *Penicillium*, but species belonging to the genera *Cephalosporium* and *Alternaria* are also known to solubilize insoluble phosphate. However, fungi are reported to be more efficient P-solubilizer than bacteria, actinomycetes and cyanobacteria (Thomas et al., 1985). Microorganisms involved in P solubilization as well as better scavenging of soluble P can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements by production of growth promoting substances (Gyaneshwar et al., 2002). Application of phosphorite

along with phosphate solubilizing bacteria (PSB) improved P-uptake by plants and yield indicating that PSB are able to solubilize phosphate and to mobilize phosphorus in crop plants (Rogers and Walfram, 1993). Phosphate solubilizing bacteria [*Pseudomonas fluorescens* Ps. (RM3M) and *Bacillus megaterium* BM (NRC 131)] inoculation in maghrabi banana (*Musa* sp.) with mineral phosphorus improved the efficiency of P fertilizer and would curtail the required P rate to a tune of 25% in plants. Improvement in vegetative growth and fruit quality, have also been reported by Attia et al. (2009). Inoculation of PSB (Microphos) along with keradix (rooting powder) found to promote fresh root development to a larger extent in stem cuttings of grape (Wange and Ranawade, 1997, 1998). The PSB-plant inoculation resulted in 10-15% increase in crop yield in 10 out of 37 experiments. Such experiments also demonstrated an increase in P-uptake by plants (Tandon, 1987). But, no correlation could be established between the pH and the degree of solubilization. Though, solubilization of insoluble phosphate could be achieved under acidic conditions (Gand and Gaur, 1990). Factors like nutrition, aeration and temperature have also great influence in phosphate solubilization (Illemer and Schinner, 1992). Narsian and Patel (1997) reported that high salt (sodium chloride) concentration badly affected P solubilization activity of *Aspergillus aculeatus*. Earlier, Gyaneshwar et al. (1998) cloned two genes responsible for conferring mineral phosphate solubilizing ability in *E. coli* from *Synechocystis* PCC 6803, a unicellular cyanobacterium. The transformants were found to solubilize rock phosphate also and the property was found to be plasmid associated. Recently, native isolates of PSB (*Pseudomonas cholorrhaphis*, *Bacillus cereus* and *P. fluorescens*) from walnut rhizosphere found to improve plant height, shoot and root dry weight and P&N uptake of walnut seedlings (Xuan Yu et al., 2011).

In fact, the improvement of P-nutrition in plants has been the most recognized beneficial effect of mycorrhiza (Bianciotto and Bonfante, 2002). The increased uptake of phosphorus following mycorrhizal inoculation has been reported in strawberry, citrus, grape, litchi, guava, banana, apple, papaya and pomegranate (Hršelova et al., 1990; Rizzardi, 1990; Antunes and Cardoso, 1991; Liang, 1995; Kon, 1995; Yamashita et al., 1998; Chacon and Cuenca, 1998; Hazarika and Ansari, 2007; Aseri et al., 2008). The mechanism that is generally accepted for such mycorrhizal role consists of a wider physical exploration of the soil by mycorrhizal fungi than by roots. Besides, hyphae that extend beyond the root depletion zone, various subsidiary mechanisms have been proposed to explain P-uptake by mycorrhizal fungi, such as the kinetics of P-uptake into hyphae differ from those of roots either through a higher affinity (lower K_m) or a lower threshold concentration at which influx equals efflux (C_{min}); root and hyphae explore microsites

differently, especially small patches of organic matter (St John et al., 1983; Jøner and Jakobsen, 1995); plant root and mycorrhizal hyphae affect chemical changes and P solubility in the rhizosphere differently. AM (*Glomus mosseae*+*Glomus fasciculatum*+*Gigaspora margarita*) inoculation in papaya with mineral phosphorus improved the efficiency of P-fertilizer and would reduce the required P-dose to plant by about 25% (Padma and Kandasamy, 1990). In corporation of easily soluble P-fertilizer or rock phosphate might feedback differently on mycorrhizal functioning. Application of soluble phosphate reduced mycorrhizal functioning in citrus rhizosphere. In contrast, addition of sparingly soluble P sources such as rock phosphate could even increase mycorrhizal colonization by 52% compared with the soluble phosphate application (Tang and He, 1991; Vanlanwe et al., 2000; Alloush and Clark, 2001). The magnitude of the effect seems to be partly crop species-specific, interacting with the extent to which rhizosphere changes affect rock phosphate dissolution.

4. Potassium Nutrition

Like N and P, potassium (K) is the third major essential nutrient required for plant growth and development. It plays an essential role for enzyme activation, protein synthesis and photosynthesis. Potassium in soil is present in water-soluble, exchangeable, non-exchangeable and structural or mineral forms. Potassium from water-soluble and exchangeable pools is directly available for plant uptake. At low levels of exchangeable-K in certain soil types, non-exchangeable-K can also contribute significantly to the plant uptake (Memon et al., 1988; Sharpley, 1989). Non-exchangeable-K consists predominantly of interlayer K of non-expanded clay minerals such as illite and lattice K in K-bearing minerals such as K-feldspars. According to Buchholz and Brown (1993) more than 98% of potassium in soil exists in the form of silicate minerals (microcline, muscovite, orthoclase biotite, feldspars, etc.). The main source of K for plant growing under natural condition comes from the weathering of K-minerals and organic K-sources such as compost and plant residues. However, it is recognized that the weathering process can be further mediated by organisms and their metabolites. Respiration by plant roots and microbial degradation of organic matter can elevate carbonic acid concentration in the soils and ground water, leading to an increase in the weathering rates of minerals (Chapelle et al., 1987; Keller and Wood, 1993). In addition to carbonic acid, direct contact between bacteria and mineral may be important in mineral alteration reaction, as microbial surface can complex with metal ions (Ferris et al., 1998; Daughney et al., 1998). It was observed that K was released from mica to water soluble and exchangeable pool of potassium due to inoculation of mica with *Bacillus mucilaginosus* in two alfisols under sudan grass cultivation (Basak and Biswas, 2008). Similarly inoculation of *Bacillus mucilaginosus*

along with K-bearing minerals was found to increase soil available K, K-uptake and promote growth of brinjal and groundnut (Han and Lee, 2005; Sugumaran and Janarthanam, 2007). Badr (2006) reported that inoculation of *Bacillus cereus* (silicate dissolving bacteria) into composting-mass appeared to enhance the percent of available K in the mature compost compared to its counterpart without inoculation. The conjunctive use of feldspar-charged-compost and *Bacillus cereus* markedly increased total K-uptake by tomato and had greater K-recovery than potassium sulphate application in sandy soil.

A strain of thermophilic fungus *Aspergillus fumigatus* was found to promote potassium release from K-bearing minerals by means of at least three likely routes, one through the complexation of soluble organic ligands, another appealing to the immobile polymers such as the insoluble components of secretion, and the third related to the mechanical forces in association with the direct physical contact between cells and mineral particles (Bin et al., 2008). Microorganisms attached to mineral surfaces can also create micro-environments where concentration of ligand, acidity and redox activity can be substantially elevated compared to the bulk solution, thus effecting mineral exchange reaction (Hiebert and Bennett, 1992; Barker and Banfield, 1996, 1998; Rogers et al., 1998; Barker et al., 1998). A number of ligands, not only oxalate but also pyruvate, citrate, succinate, malate, gluconate, lactate and fumarate have been detected in soils and on weathered rocks colonized by bacteria and fungi (Fox and Comeford, 1991; Palmer et al., 1991; Baziramakenga et al., 1995; Krzyszowska et al., 1996).

Arbuscular mycorrhizal fungi have also been reported to improve K-nutrition in plant. Concentrations of K were higher in mycorrhizal than non-mycorrhizal plants (Bressan et al., 2001; Liu et al., 2002). Increased K concentration can be a consequence of increased P availability on plant growth and the effects of mycorrhizas on P and K are laborious to disentangle. Thus, bio-intervention on K-bearing minerals could be an alternative and viable technology to solubilize insoluble K into soluble form and could be used effectively as a source of K-fertilizer for sustaining fruit crop production and maintaining soil potassium.

5. Micro-nutrient Nutrition

Micronutrient deficiencies, especially those of zinc (Zn) and iron (Fe), are of major concern in developing countries causing especially serious health problems in human especially in infants, children and women. Zn and Fe deficiency in humans is a consequence of the limited bio-availability of Zn and Fe in tropical soils and hence in plants. Problems may have been exacerbated as a consequence of increased fertilizer use. Increased crop production through application of macronutrient



fertilizers can dilute Zn and Fe concentrations in plants. This is the well-known dilution effect, increased macronutrients availability improve plant growth and thereby spreads other available nutrients throughout plant tissue. There is no dearth of literature pertaining to AM fungi indicating enhanced micronutrient uptake particularly Zn and to some extent Fe and Cu in various fruit crops. AM inoculations have been found to increase leaf Zn, Fe and Cu content in citrus, peach and Ottawa apple over non-inoculated plants (Viyanak and Bagyaraj, 1990; Singh and Sharma, 1993; Venter and Thomas, 1993; Godara et al., 1996). Though, the effect was more pronounced with dual inoculation of VAM and *Azotobacter*. In general, AM fungi are involved in the interaction between P and Zn. The application of P fertilizer found to reduce mycorrhizal functioning and led to a lower uptake of Zn. A similar response was also demonstrated by Lambert et al. (1979). Mehravaran et al. (2000) provided evidence that different species of AM fungi differ for the uptake effectiveness of P and Zn, because uptake of both elements in mycorrhizal plants was not correlated. A possible explanation is that AM fungi differently express P and Zn transporters or differently down-regulate the P and Zn transporters of plants. Treeby (1992) also reported the role of VAM inoculation in improving Fe-nutrition in citrus under acidic soil condition. However, in many environments the amount of soluble iron is too low to sustain the microorganisms. This low solubility and hence bioavailability of iron is overcome by some organisms through the production and excretion of iron-chelating compounds, termed siderophores. These compounds bind with iron to form a siderophore-iron complex which is then taken up by the cell and iron is later liberated internally (Gram et al., 2002). In particular, the so-called fluorescent pseudomonads *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* produce a water-soluble yellow-green fluorescent (under UV light) pigment called pyoverdine (Meldrum, 1999). This pigment is responsible for the characteristic fluorescence of the cell and has also been identified as an iron-chelating siderophore (Meyer and Abdallah, 1978; Neilands, 1983; Fernandez et al., 1988). It has been observed that pyoverdine production is stimulated by conditions of iron limitation, and its production decreases with increasing iron availability (Meyer and Abdallah, 1978). Dual inoculation with *Pseudomonas fluorescens* and VAM increased ferrous iron and leaf chlorophyll contents in the most lime induced chlorosis susceptible grape rootstock in calcareous soil and higher mycorrhizal colonization also enhanced Mn and Cu concentration in leaf fresh matter (Bavaresco and Fogher, 1992, 1996ab).

Organic acids released in the surrounding medium by a range of ericoid mycorrhizal fungi can solubilize insoluble inorganic metal compounds, and suggests that isolates from polluted and unpolluted environments differently influence Zn mobility. In the terrestrial environment, solubilization of insoluble

metal compounds is important for the release of trace metals as well as associated anionic nutrients e.g. phosphate into biogeochemical cycles (Gadd, 1993, 1999; Morley et al., 1996). This appears to depend mainly on the excretion of various metabolites, including organic acids, and protons (Franz et al., 1991; Burgstaller and Schinner, 1993; Sayer et al., 1995; Gadd, 1999). The *Oidiodendron maius* isolates produced fumarate, malate and citrate that solubilized the insoluble inorganic Zn compounds. The amount of organic acids released by the fungi into the medium could be correlated, at least for a limited number of *O. maius* fungal isolates, with the solubilization activity shown in Petri dishes. Organic acids perform a diverse range of functions in the soil, and most of these processes appear to be beneficial to resident organisms (Jones, 1998). Depending on the number and dissociation properties of their carboxylic groups, they can complex metal cations to different degrees, and displace anions from the soil matrix. Citrate and malate in particular are strong natural chelators of trivalent cations such as Al^{3+} and Fe^{3+} , and are involved in the scavenging of metal ions from insoluble compounds in the soil. Particularly citrate has been shown to be the most important Al^{3+} complex former in soil solution from podzolised forest soils (Van Hees et al., 2001). These organic acids are released in the soil by bacteria, fungi and plant roots (Jones, 1998). Their production in fungi has been reported to increase at growth limiting concentrations of soluble metal ions such as Mn^{2+} , Fe^{2+} and Zn^{2+} (Gadd, 1999). The nature and amount of organic acids excreted by fungi can be strongly influenced by the pH and the buffering capacity of the medium, the carbon source and the nitrogen-phosphate balance (Kubicek et al., 1988). Also, Burgstaller and Schinner (1993) reported that the production of citric acid by *Penicillium simplicissimum* was induced after adsorption of ZnO on the mycelium, probably because of a change in the membrane H^+ -ATPase activity.

6. Plant Growth Promotion

Over the past two decades, bacteria from the plant's rhizosphere have received considerable attention with respect to plant growth promotion. However, rhizosphere bacteria are collectively called plant growth promoting rhizobacteria (PGPR). These bacteria vary in their mechanism of plant growth promotion but generally influence growth via phosphate solubilization, nutrient uptake enhancement or producing plant growth hormone. Even, inoculation of *Azospirillum* along with nitrogenous fertilizers found to enhance growth parameters like height, girth of stem and leaf area and also increased yield in banana and strawberry (Jeeva et al., 1988; Wange, 1996). It has also been demonstrated that *Azospirillum* inoculation along with recommended dose of fertilizer increased yield significantly in pumpkin, potato and onion (Mahendran and Kumar, 1998; Karuthamani et al., 1995; Thilakavathy and

Ramaswamy, 1999; Nanthakumar and Veeraragavathatham, 1999). Also Mahmoud and Mahmoud (1999) showed that the treatment of peach seedling with *Azotobacter chroococcum* and *Bacillus megatarium* led to increase in the plant height, stem diameter, leaf number, plant dry weight, leaf area, leaf micronutrient and chlorophyll content. Inoculation of mango seedlings with *Glomus fasciculatum* and *Azotobacter chroococcum* strain 1 had increased seedling height, diameter, leaf area, and total root length under solarization and black polyethylene mulching (Sharma et al., 2011). In pomegranate *Trichoderma harzianum* was used which could promote the rooting in its stem cuttings (Satish Kumar et al., 2001). There are reports that few microorganisms have been used for acclimatization and growth promotion in micro-propagated fruit plants. Earlier, Ruiz (1997) showed that inoculation of micropropagated banana plantlet with mycorrhizal strains (*Glomus fasciculatum*, *Acaulospora escrobiculata* and *Glomus mexicanum*) and treatment with phosphorine bio-fertilizer resulted in increased dry weight of plant and reduced hardening period. Similarly, Mathur and Vyas (1999) reported that the *in-vitro* multiplied VAM strain established efficient symbiosis with *in-vitro* raised plantlets of *Ziziphus mauritiana*. Use of such VAM strain found to improve the biomass production, nutrient uptake and acclimatization of *in-vitro* produced *Ziziphus mauritiana* plantlets in the field. An artificial symbiosis between *Azotobacter vinelandii* and strawberry callus was successfully established through using biolistic gum (Preininger et al., 1996, 1997). A tripartite culture system (wherein micro-propagated strawberry plantlets, after root induction grown on cellulose plug were placed in contact with the primary mycorrhizae in growth chambers enriched with 5000 ppm CO₂ and fed with minimal medium) was also successful in establishing symbiosis with micro-propagated strawberry plantlets. It was observed that the VAM symbiosis reduced osmotic potential of plantlets. This response might be a useful pre-adaptation for plantlet during transfer to the acclimatization stage (Elmeskaoui et al., 1995). The studies on role of mycorrhizal inoculation on post acclimatization growth of micro-propagated fruit plant indicated that the growth and development of *Annona cherimola* plant profited from mycorrhizal establishment. Most of the *Glomus* sp. markedly increased shoot, root biomass and leaf area of micro-propagated plant. The greatest effect of VAM fungi on plant growth were observed when they were introduced after the acclimatization period (Azcon-Aguilar et al., 1994). So, micro-propagated *Annona cherimola* plants are more dependent on mycorrhiza formation for optimum growth than plant derived from seeds. Similarly, VAM inoculation significantly increased the fruit yield in strawberry. It was demonstrated that the combination of strawberry cultivar and endophyte also play role in maximizing benefit obtained from mycorrhizal colonization (Chavez and Ferrera Cerrato, 1990). Further, under green-house condition

VAM fungi inoculation was found to promote a good growth in orange and peach seedlings (Chang and Chien, 1990; Xue and Luo, 1992). It was reported that *cytokinin* activity in roots and leaves was associated with differences in seedling total dry weight and VAM colonization. Moreover, seedling leaf tissue had greater *cytokinin* activity than root tissue. Interestingly, VAM fungi colonization in root of fruit plant is reported to have beneficial effect on plant growth even under stress condition. Under high air temperature stress condition the photosynthesis and transpiration rates of VAM inoculated Satsuma mandarin trees were higher to those of non-VAM ones. Though, VAM-trees had three times more photo-assimilates per tree than uninfected ones as the VAM infected trees had leaf area three times larger than the uninfected trees which grew more vigorously.

7. Conclusion

The role of various root associated microorganisms in plant nutrition and growth as well as make use of their potential beneficial features as bio-fertilizers in horticultural crop production has been presented with few examples of bacteria and fungi, which may bear a great promise as bio-fertilizers. In order to harness the potential benefits derived from these organisms, more information is urgently needed on the interactions among plants and rhizosphere associated microorganisms. Various fungi and bacteria provide a battery of extracellular enzymes, which could be utilized for improving crop yields and curtailing costs of inorganic fertilizers. Thus, multiple organisms' inoculation may be of great value, if various organisms with different proven or suspected benefits to the crop plant can be integrated for sustainable production and eco-friendly environment.

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