About TERI

TERI is a an autonomous not-for-profit, research institute established in 1974 and is involved in research activities in the fields of energy, environment, biotechnology, forestry, and the whole range of sustainable development issues. More specifically, its activities are geared to promoting large-scale use of renewable forms of energy, protecting the earth's ozone layer, mitigating the threat of climate change, and reversing the loss of forest cover and biodiversity, all of which transcend national boundaries and require solutions through global cooperation.

About the Biotechnology Division

The Biotechnology Division seeks to achieve sustainable development through wide-ranging research, from microbes on the one hand to giant forest trees on the other. The Division has three areas: Microbial Biotechnology, Plant Molecular Biology, and Plant Tissue Culture.

The Division also incorporates the Asian Network on Mycorrhizae which coordinates research activities in the field in Asia. A National Facility on Germplasm of Mycorrhizal Fungi has also been established. It has been demonstrated that these organisms are efficient tools for growth enhancers and reclamation of barren sites overburdened with fly ash.

About Mycorrhiza Network and CMCC...

The Mycorrhiza Network, located at the Tata Energy Research Institute (TERI), New Delhi, was set up in 1988 with an important component—the Mycorrhiza Information Centre (MIC). With effect from December 1993, a germplasm bank facility named Centre for Mycorrhizal Culture Collection (CMCC) started functioning as a composite project with the Mycorrhiza Network. The general objectives of the Mycorrhiza Network are to strengthen research, encourage cooperation, promote exchange of information, and publish a quarterly newsletter, Mycorrhiza News. The MIC is primarily responsible for establishing an information network in the region that makes available to the researchers the growing literature on mycorrhiza and facilitates information sharing among the members. A database on mycorrhizal literature is operational for information retrieval and supply to researchers, on request.

The main objectives of the CMCC are to procure strains of both ecto and VA mycorrhizal fungi from India and abroad; multiply and maintain these fungi in pure culture; screen, isolate, identify, multiply, and maintain native mycorrhizal fungi; develop a database on cultures maintained at CMCC and provide starter cultures on request. Cultures from CMCC are available on an exchange basis or on specific requests at nominal costs for spore extraction/handling, etc.

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Interaction of mycorrhizae with soil microflora and microfauna—Part 1. Interaction with soil microflora (except soil microfauna and free living nitrogen fixers)[1]

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Soil microflora mostly consists of free living micro-organisms in soil and specific microbial communities in rhizosphere and rhizoplane regions. All these micro-organisms which include fungi, actinomycetes, plant growth promoting rhizobacteria, soil pseudomonads, chitin decomposing bacteria, acid producing bacteria, and phosphorus solubilizing bacteria, among others, affect mycorrhizal development on plant roots and the survival of mycorrhizal fungi in soil in one way or the other. These organisms may stimulate or inhibit the development of mycorrhizae on plant roots, may not have any effect or may even parasitize mycorrhizae or fruit bodies of mycorrhizal fungi, thus, affecting their efficiency. The following account deals with such varied types of interactions between mycorrhizae and associated soil microflora.

Soil microflora associated with mycorrhizal roots

Qualitative and quantitative studies conducted at the University of Agricultural Sciences, Bangalore, India, showed that the fungal populations associated with the root zones of VA-mycorrhizal pot cultures of *Glomus fasciculatum*, *Gigaspora margarita*, *Acaulospora laevis*, and *Sclerocystis dussii* were not altered, except with *S. dussii*, where a significant decrease was recorded. *Cladosporium herbarum* was the predominant species associated with all the VA-mycorrhizal pot cultures (54). Further studies conducted at the above University showed that the total bacterial populations, number of nitrogen fixers, and gram negative bacteria were significantly higher in pot cultures of *Glomus fasciculatum*, *Gigaspora margarita*, and *Sclerocystis dussii* than in control without VA-mycorrhizal fungi. Spore formers decreased and urea hydrolysers increased in the above three cultures and in pot cultures of *Acaulospora laevis* which harboured fewer bacteria than controls [without vesicular–arbuscular mycorrhiza (VAM)]. The occurrence of the amino acid requiring bacteria increased in all the pot cultures except in *A. laevis*. Pot cultures of *G. fasciculatum* and *A. laevis* had significantly more actinomycetes antagonistic to the pathogens *Fusarium solani* and *Pseudomonas solanacearum* and those of *G. margarita* had more actinomycetes antagonistic to phytopathogen *Xanthomonas campestris* pv. *Vignicola* as compared to controls (53). Studies conducted at the University of Bayreuth, West Germany, revealed that the total number of bacteria but not actinomycetes on the rhizoplane of *Zea mays* and *Trifolium subterraneum* increased in plants infected by *Glomus fasciculatum* compared with plants without VAM. In the rhizosphere soil of VAM plants, however, the populations of bacteria and actinomycetes were not affected quantitatively but the VAM affected specific groups of bacteria and actinomycetes in both the rhizosphere soil and the rhizosphere. The rhizosphere soil

[1] Compiled from TERI database—Riza

of mycorrhizal plants contained more facultative anaerobic bacteria and fewer fluorescent pseudomonads but had the same number of gram negative bacteria as non-mycorrhizal plants. Among actinomycetes, populations of both *Streptomyces* sp. and chitinase producing actinomycetes decreased in the rhizosphere but not in the rhizoplane of mycorrhizal plants (37). Studies conducted at the University of Delhi, India, showed that the effect of mycorrhiza on soil fungi was more pronounced on the root surface of cotton plants than in the rhizosphere. Several pathogenic fungi like species of *Fusarium, Rhizoctonia, Pythium*, and *Verticillium* were found to have higher frequency of occurrence on the rhizosphere and rhizoplane of non-mycorrhizal plants as compared to root-zone fungi of mycorrhizal plants. Several phosphorus solubilizing fungi like *Aspergillus* spp. and other saprophytes were found to be higher in number on mycorrhizal roots as compared to non-mycorrhizal roots (35). In further studies at the above university, six combinations of VAM fungi and phosphorus fertilizer treatments were applied to *Leucaena leucocephala* roots and quantitative and qualitative observations were made periodically of the rhizosphere microflora and constituents of root exudates. The results indicated that the presence of specific microflora in the rhizosphere of mycorrhizal roots is mediated through root exudates rather than being an outcome of improved phosphorus nutrition (9). Studies conducted at the Rajendra Agricultural University, Bihar, India, on different varieties of *Litchi chinensis* showed that rhizosphere of the cultivars Kasva, Deshi, and Purbi infected with vesicular–arbuscular phycomycete, *Rhizophagus*, had higher populations of bacteria and protozoa and these populations differed not only from non-rhizosphere soils but also with mycorrhizal formation in different cultivars of *L. chinensis* (45).

In studies conducted at the University of Western Australia, Nedlands, Australia, endotrophic bacteria isolated from the mycorrhizal tissues of 12 species of Western Australian terrestrial orchids were placed into eight groups based on UV, light fluorescence, gram staining, and colony characteristics. Most commonly isolated bacteria from 9 out of 122 orchid species sampled were strains belonging to *Pseudomonas fluorescens-putida* group. Abundance of bacteria followed a seasonal pattern that differed between orchid genera especially on the basis of the morphology of the fungus-infected tissues. There was little evidence of specificity of the bacterial groups to orchid taxa or part of the plant infected by the fungus.

Symbiotic germination of *Pterostylis vittata* seed, in association with seven bacterial isolates, showed a significant promotion of germination and seedling development with three bacterial strains, suppression of seedling development with other three bacterial strains while having no effect on the remaining one bacterial strain (64). In studies conducted at the United States Department of Agriculture–Agricultural Research Service (USDA–ARS), Horticultural Research Laboratory, Camden Rd, Orlando, Florida, on greenhouse pot cultures of *Citrus reticulata*, the soil was amended with either *Glomus intraradix* or 630 µg phosphorus per g of soil or both or kept unamended as control. Most microflora groups increased in rhizosphere of most treatments when compared to non-rhizosphere soil. Gram negative bacteria, protein hydrolysers, anaerobic bacteria (in one of the two treatments) and fluorescent pseudomonad (in one of the two treatments) did not differ in rhizospheres of VAM alone, phosphorus alone, and unamended control treatments. Fluorescent pseudomonads and actinomycetes in one of the two tests were significantly higher in treatment with VAM alone than in control treatments. Treatment with phosphorus alone and phosphorus with VAM treatment influenced the population of some microflora of the rhizosphere more than the treatment with VAM alone (41). Studies conducted at the Laboratory of Microbiology, Institute of Biology, Nicolaus Copernicus University, Torun, Poland, showed that all bacteria and actinomycetes associated with mycorrhizae of *Porus sylvestris* produced auxins (in medium containing tryptophan), vitamins (biotin being produced in the smallest and thiamine in the largest amounts) and excreted glutamic acid, alanine, lysine, and valine. In addition, the bacteria were capable of producing cytokinin-like substances and releasing different organic acids. No bacterial strain exhibited proteolytic activity but some showed cellulolytic activity (58).

Stimulating effect of general soil microflora on mycorrhizal development

Micro-organisms belonging to a wide range of taxonomic groups are known to stimulate the establishment and stability of mycorrhizal symbiosis. The positive interactions may be due to direct trophic effect, detoxification or indirect effect through action on the root and antagonism to other micro-organisms inhibiting the mycorrhizal development (42). In studies conducted at the Institut National de la Recherché Agronomique, Champenoux, France, two mechanisms were considered in stimulating the effect of some soil bacteria on the growth of two ectomycorrhizal fungi (*Hebeloma crustuliniforme* and *Paxillus involutus*). These were: (i) a direct trophic effect of the bacteria on the fungi and (ii) an indirect effect of detoxification of the fungal culture

medium.

This was supported by the observations that: (i) some organic acids (citric acid, and malic acid) released by the bacteria stimulated the growth of the two fungi and (ii) *P. involutus* released polyphenolic substances that are toxic in themselves but are metabolized by the bacteria. These results suggested that some bacteria could have a beneficial effect during the saprophytic phase of ectomycorrhizal fungi before mycorrhizal infection (16). Studies conducted at the Commonwealth Scientific and Industrial Organization, Glen, Osmond, Australia, showed that the addition of general soil microflora from four soils to autoclaved soil, in which *Pinus radiata* seedlings had been raised, either enhanced or impeded the mycorrhizal development by *Rhizopogon luteolus, Paxillus involutus*, and *Hebeloma crustuliniforme* depending upon the soil on which the plants were grown and on the mycorrhizal fungus; the enhancement being more frequent than reduction (18). Studies conducted at the Universite Laval, Quebec, Canada, on effects of vegetative inoculum of *Laccaria bicolor, Pisolithus tinctorius, Hebeloma cylindrosporum*, and fine soil suspension on mycorrhizal development in *Pinus banksiana* and *Larix laricina* seedlings showed that fine soil plus *L. bicolor* inoculum formed significantly more mycorrhiza in pine seedlings than other combinations of the inocula (mixed inoculum of all three fungi or mixed inoculum of *L. bicolor* and *H. crustuliniforme*). In *L. laricina*, however, inoculation with *L. bicolor* formed significantly more mycorrhiza than other types of inocula. The addition of fine soil suspension containing biological propagules less than 45 µm enhanced mycorrhiza formation and influenced shoot length and dry weights with some of the tested inocula, depending on the tree species. The competitive ability of the symbionts in the mixed inoculum was also affected by the soil suspension (38). Studies conducted at the Central Institute of Medicinal and Aromatic Plants, Lucknow, India, showed that the level of infection by *Glomus* spp. was 44.6% in healthy *Cymbopogon flexuosus* plants but when these plants were infected with *Balansia sclerotica*, the level of infection was enhanced to 89.6% (24). Studies conducted at the Justus-Liebig University, Giessen, Germany, showed that although *Trichoderma* spp. have a high antagonistic potential towards numerous fungi, infection and colonization of maize roots by autochthonous VAM fungi in a field soil, with or without additional inoculum with *Glomus etunicatum,* were almost unaffected by a strain each of *Trichoderma hamatum* and *T. harzianum*, suggesting that a combined application of VAM and *Trichoderma* spp. to promote plant growth should be feasible (26). Studies conducted at the Centre d' Investigacion, Agraria de Cibrils, Spain, on two *Trichoderma aureoviride* and two *T. harzianum* isolates for their effect on *in vitro* germination of *Glomus mosseae* spores showed that inoculation with each isolate separately had no significant effect on resting spore germination but there was a highly significant increase in the production of vegetative spores when both *Trichoderma* spp. were inoculated at the centre of the plate (10).

Inhibitory effect of general soil microflora on mycorrhizal development

Studies were conducted at the OMH Laboratory, Services Branch, Toronto, Canada, on ten mycorrhizal isolates from the rhizosphere of mycorrhizae of black spruce for their ability to inhibit the formation of mycorrhiza on axenically cultivated black spruce seedlings by *Laccaria bicolor*. The results showed that *Trichoderma viride* and *T. polysporum* were strongly antagonistic towards mycorrhizal colonization both when *L. bicolor* and *Trichoderma* spp. were inoculated together or if mycorrhizal fungus was allowed to establish in the rhizosphere first and then inoculated with *Trichoderma* spp. However, despite its antagonism in flask trials, *T. viride*, did not parasitize the hyphae of *L. bicolor* in agar culture. Two other fungi, *Tolypocladium inflatum* and *Trichosporon beigelii*, also had small but significant inhibitory effects on the formation of mycorrhizae in the flask trials. However, a variety of Mucoralean fungi, heavily sporulating Hyphomycetes, and non-sporulating root- associated Hyphomycetes had no deleterious effect on this process (60).

Interaction of mycorrhizal fungi with rhizosphere microflora

Studies conducted at the Universitat Mohenheim, Stuttgart, Germany, showed that *Zea mays* plants grown on calcareous soil in pots divided by 30 µm nylon nets into three compartments (the centre for root growth and the outer for hyphal growth) and inoculated with rhizosphere micro-organisms and VAM fungus, *Glomus mosseae*, had higher shoot-root ratios; significantly increased concentration of phosphorus, zinc, and copper; and decreased the concentration of manganese in roots and shoots than in control plants (with a gamma-irradiated inoculum) and the plants inoculated with rhizosphere micro-organisms only after three or six weeks' growth. Root exudates were collected on agar sheets placed on the interface between root and hyphal compartments. Six-week old VAM and non-VAM plants had similar root exudate compositions of 72–73% reduc-

ing sugars, 17–18% phenolics, 7% organic acids, and 3% aminoacids. However, three- to sixfold higher amounts of carbohydrates, aminoacids, and phenolics were recovered when antibiotics were added to agar sheets on which root exudates were recovered.

Thus, the high microbial activity in the rhizosphere and on the rhizoplane limits the exudates recovered from the roots (5). Studies were conducted at the Estacion Experimental Zaidin, Consejo Superior de Investigaceones Cientificas (CSIC), Granada, Spain, to test a selective interaction between free living rhizosphere flora and VAM fungi in experiments conducted on tomato (*Lycopersicum esculentum*) plants raised in sand-vermiculite medium and inoculated with two rhizosphere bacteria A and P and three VAM fungi namely *Glomus mosseae, G. fasciculatum*, and *Glomus* sp. (E3 type). Generally, bacterial inoculation increased the growth of mycorrhizal plants. Plants inoculated with *G. fasciculatum* showed increased growth with A or P or A+P while plants inoculated with *G. mosseae* did not show any response to A and in plants inoculated with *Glomus* sp., treatment with P did not increase plant growth. Germination, hyphal growth, and vegetative spore production of *G. mosseae* spores cultivated *in vitro* under axenic conditions were increased in the presence of A or P; P being more effective than A (6).

Further studies at the same place on pot experiments to determine the effect of two free-living micro-organisms, a bacterium (B) and a fungus (F) on different stages of development of *Glomus mosseae* and *G. fasciculatum* in lucerne showed that both F and B increased root colonization by *G. mosseae* and both decreased the establishment of *G. fasciculatum* but B and F increased the number of spores from both mycorrhizal inocula. The results confirm that soil rhizosphere micro-organisms–VAM fungus interactions occur and are dependent on the pair combination (8). Studies conducted at the University of Hanover, Germany, showed that several bacterial isolates from the rhizosphere, particularly an isolate of *Bacillus cereus*, improved the frequency but not the intensity of VAM in flax. Using bacteria with the commercial inoculum of VAM in the same clay particle, mycorrhization was enhanced in the eight crop species tested and with three VAM isolates. Soil quality had a limiting influence. However, with the increasing amount of compost in the sand, the effects of bacteria disappeared (1).

Effect of plant growth promoting rhizobacteria on mycorrhizal development

Studies conducted at the Institut Agronomico Campinas, Brazil, on beans showed that inoculation with both *Glomus etunicatum* and plant growth promoting rhizobacteria (fluorescent pseudomonads) resulted in the increase in root growth, nodulation, uptake, and use efficiency of nitrogen and phosphorus (56). Studies were conducted at the Horticultural Research Laboratory, Corvallis, USA, on the interaction of an indigenous VAM fungus with plant growth promoting rhizobacteria (PGPR), *Pseudomonas putida*, on *Trifolium subterraneum*, grown on non-sterile soil. The results showed that at 12 weeks growth, plant growth, root dry weight, shoot dry weight, nodulation, and concentration of iron, copper, aluminium, zinc, cobalt, and nickel in shoots were significantly greater with PGPR + VAM inoculation over PGPR alone, VAM alone or control though these growth parameters were also greater with PGPR alone or VAM alone inoculation when compared to control. Inoculation with PGPR increased VAM colonization from 7% to 23% at six weeks but colonization levels with VAM fungi were similar (approximately 50%) at 12 weeks. Populations of PGPR increased similarly in the rhizosphere of both mycorrhizal and non-mycorrhizal plants (36). Another study conducted at the University of British Columbia, Vancouver, Canada, on *Pinus contorta* seedlings grown with 0.35 mM Ca $(^{15}NO_3)_2$ as the only nitrogen source showed that inoculation with *Wilcoxina mikolae* (isolate R947) resulted in the synthesis of ectendomycorrhizae but decreased shoot biomass and total foliar nitrogen though root biomass and shoot height were not affected. Co-inoculation of seedlings with *W. mikolae* and PGPR (*Bacillus* strain 6), capable of fixing nitrogen, resulted in a similar degree of mycorrhization, shoot biomass was greater as compared to seedlings inoculated with *W. mikolae* alone but root biomass and stem height were not altered. Total foliar nitrogen was lower in seedlings inoculated with *Wilcoxina* and co-inoculated plants. Bacterial inoculation alone did not affect seedling biomass or foliar nitrogen content. Based on the 15 N to 14 N ratio of foliage, associative nitrogen fixation by *Bacillus* contributed four per cent of the seedling foliar nitrogen whether or not seedlings were mycorrhizal. These results suggest that growth of mycorrhizal pine can be stimulated by inoculation with beneficial bacteria. Growth promotion does not result from increased formation of ectendomycorrhiza and may, only in part, result from associated nitrogen fixation (14). Further studies at the above University on Rifamycin-resistant derivatives of plant growth promoting *Bacillus polymyxa* strains L6, Pw2, and S20 showed that these are the three strains which stimulated growth of pine and spruce seedlings but this

stimulation was similar for both mycorrhizal and non-mycorrhizal seedlings of both species. Bacterial inoculation did not influence the mycorrhizal status of the seedlings (55). Studies conducted at the Horticultural Research Laboratory, Corvallis, USA, on cucumber seeds treated with rifampin-resistant derivatives of *Pseudomonas putida* (A12, N1R, or R20) and *P. fluorescens* (2-79 or 3871) and planted in soil with or without inoculum of *Glomus intraradices* or *G. etunicatum* showed that populations of all the strains except R20 at one to three weeks as determined by dilution plating on selective medium were 1.5–7 times lower in the rhizosphere of cucumber roots colonized by *G. intraradices* as compared to non-mycorrhizal plants but the effect was less consistent in three to nine-week old plants. No significant difference was detected in *G. etunicatum* inoculated plants. Antibiotic producing strains 3871 and 2–79 delayed the germination of *G. etunicatum* spores but by seven days no significant difference in frequency of germination was detected. None of the *Pseudomonas* strains affected the colonization of cucumber roots by *G. etunicatum* as determined by measures of mycorrhizal inoculum density–root colonization relationships (47). Studies conducted at the Texas Technical University, Lubbock, Texas, USA, on rice plants at the pretransplant/nursery stage showed that dual inoculation of the soil with VAM fungi and fluorescent *Pseudomonas* spp. produced plants with highest biomass, and intermediate root length as compared to increased shoot growth, root length, and fine root mass and decreased root growth and phosphorus and nitrogen concentration in only Pseudomonas inoculated plants and reverse of the above in the VAM inoculated plants. Mycorrhizal colonization percentage and colonized root lengths were significantly lower in dual inoculated plants as compared to plants inoculated with VAM alone. It is suggested that VAM colonization is suppressed by the presence of fluorescent *Pseudomonas* spp. resulting in a reduced photosynthate loss from the plant to the fungi and a corresponding increase in plant biomass and increased nutrient levels (15).

Studies conducted at the University of Saskatchewan, Saskatoon, Saskatchewan, Canada, on interactions between plant growth-promoting fluorescent *Pseudomonas* sp. and *Glomus* sp. (NT4, INVAM No. SR 101), in laboratory and in growth chambers, showed that germination of *Glomus* spores was inhibited when incubated either on 0.22 µm polycarbonate membrane placed directly on the lawn of *Pseudomonas cepacia* (R85) and *P. putida* (R104) (both known for their antibiosis against plant pathogens *in vitro*) or on agarose blocks (3 mm thick) separated from the bacteria by 0.22 µm polycarbonate membrane. Germination was not inhibited when agarose blocks were separated from the bacteria by a glass slide indicating involvement of a non-volatile, diffusible substance(s). In *Triticum aestivum* plants raised in sterile, *Leonard jars*, *Glomus* sp. (NT4) enhanced root growth in some instances but did not benefit shoot growth. In non-sterile pots, root growth was enhanced in some cases but reduced shoot and total dry weight and seed yield were observed. Dual inoculation with Pseudomonads limited or alleviated the growth depression associated with *Glomus* sp. Plant growth response to Pseudomonads varied from beneficial to detrimental and *Glomus* sp. typically nullified the detrimental effects but limited beneficial plant growth responses attributable to Pseudomonads. Synergism between fluorescent Pseudomonads and *Glomus* sp. was not detected (62). Studies conducted at the Station de Recherché sur les Champignons, Cedex, France, on *Corylus avellana* (hazel) seedlings inoculated with *Tuber melanosporum* and grown in pots inoculated with fluorescent Pseudomonads showed that *Pseudomonas fluorescens* and *P. putida* depressed *T. melanosporum* infection although the infection subsequently recovered to original levels. In plants without Pseudomonads *T. melanosporum* infection remained constant throughout the experiment. Infection of hazel by mycorrhizal competitors declined in Pseudomonad inoculated plants throughout the experiment and was significantly less (P 0.001) than in control plants at 12 months. All bacterial strains tested produced similar results although some were more efficient than the others. The protective effect of Pseudomonads against soilborne mycorrhizal competitors and their use in nurseries may be considered (34).

Studies conducted at the Estacion Experimental del Zaidin, CSIC, Granada, Spain, on *Pisum sativum* L. showed that the rhizobacterium, *Bacillus* sp., strain BH11, had no significant effect on plant biomass production in plants grown both on gray silt loam soil of high phosphorus and a yellow clay loam soil of low phosphorus content in the absence of VAM fungus, *Glomus mosseae*, but decreased plant growth by more than 30% in the presence of VAM fungus. The rhizobacterium, however, enhanced the root/shoot and seed/plant ratios in plants with both VAM and non-VAM treatments. Both the soils disaggregated in the absence of VAM fungus and the rhizobacterium did not affect the process significantly. This slaking of water-soluble aggregation increased up to 27% in the absence of VAM in the above experiment (4).

Studies conducted at the Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, India, on *Morus alba* grown with

two levels of nitrogen and phosphorus showed that combined inoculation of plants with plant growth promoting rhizobacteria (*Pseudomonas fluorescens*), *Glomus fasciculation* and *Azospirillum* were superior to dual or single inoculants or uninoculated controls and enhanced the quality of mulberry leaves and consequently resulted in the increase of all economically important characters of silkworm larvae and silk production (48). Studies conducted at the Escola Superior de Agriculture de Lavaras, Brazil, and the Institute for Tropical and Subtropical Crop Science, University of Cottingen, West Germany, on cassava showed positive effect on growth, uptake of nutrients, and sporulation of the VAM fungus with simultaneous inoculation of plants with *Acaulospora longula* and *Pseudomonas putida*. These effects were absent in cassava plants which were simultaneously inoculated with *P. putida* and *Glomus manihotus* or *Entrophosopra colombiana*. Also, in cassava not inoculated with VAM fungi, weak or no development of *P. putida* was observed on the rhizoplane (43). Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, Bangalore, India, on cow-pea showed that out of three mycorrhiza helper bacteria studied, H7 and S3 isolates enhanced per cent mycorrhizal colonization, total plant biomass, and total phosphorus contents of the plants (33). Studies conducted at the Division of Environmental Sciences, Indian Agricultural Research Institute, New Delhi, India, showed that fluorescent pseudomonads (PGPRs) and *Bacillus* spp can synergistically interact with indigenous VAM fungi for increasing growth and economic yield of wheat plants (27).

Interactions between mycorrhiza and phosphorus solubilizing bacteria

On tree crops

In studies conducted at the National Chung Hsing University, Taichung, Taiwan, there was an average of 33.2% increase in growth in *Leucaena leucocephala* seedlings by inoculation with phosphorus solubilizing bacteria (PSB) in five out of six treatments with and without sterilized soil. There was 22–99% increase in VAM inoculated plants in three subtropical/tropical soils. A synergistic effect on the growth of *L. leucocephala* occurred with PSB + VAM inoculation on unsterilized soil. Mixed inoculation with PSB + VAM also promoted the growth of *Acacia confusa* (14–63%), *A. mangium* (7–88%) and *Liquidamber formosana* (24–280%) in three tested soils. The beneficial effect of PSB can be demonstrated in soils with a high content of available phosphorus (95 µg/g soil) (65). Studies conducted at the CNRS Centre, de Pedologie Biologique, Cedex, France, showed that in a soil-vermiculite mixture without phosphorus, mycorrhizal association between *Pinus caribaea* and *Pisolithus tinctorius* and inoculation with *Bacillus* sp. stimulated the growth of *Pinus caribaea* but not significantly. But addition of tricalcium phosphate significantly increased plant growth and plant phosphorus content. Addition of organic phosphate (phytate) with bacterium alone or mycorrhiza alone had no significant effect on plant growth and phosphorus uptake but with dual inoculation, plant growth and mobilization and uptake of phosphorus were significantly increased (12). Another study was conducted at the above place to study the weathering of minerals under the influence of *Laccaria laccata* and two phosphate-dissolving bacteria (*Agrobacterium radiobacter* or *Agrobacterium* or *Achromobacter* sp.) in the rhizosphere of *Pinus sylvestris* (pine) and *Fagus sylvatica* (beech) using small lysimeter columns filled with sand, rock phosphate, and phyllosilicates. The use of lysimeters allowed construction of a balance sheet of elements released by minerals and leached or absorbed by the roots. The growth of both pine and beech increased under the influence of phosphate dissolving bacteria. *L. laccata* increased growth only in beech. Leachate acidification was greater with pine seedlings than with beech seedlings and greater in beech when inoculated with *A. radiobacter*. In dual inoculation with the fungus and one of the bacterium, no synergistic effect was observed either on mineral element release in leachate or on the plant growth (30).

In another study conducted at the above place, *Fagus sylvatica* plants inoculated or uninoculated with *Laccaria laccata* and/or a PSB, *Agrobacterium radiobacter*, were cultivated in lysimeter cylinders containing rock phosphate and a mica (phlogopite) as the only source of P, Fe, Mg, and Al. After two years, plant dry matter; P, Mg, Fe, and K uptake; and mineral element mobilization from rock phosphate or phlogopite was higher with PSB or *L. laccata* but not with dual inoculation of PSB plus *L. laccata* than in uninoculated plants. Such mineral element dissolution can be related to the increased root growth and increased amount of organic acids. The absence of synergistic effect in dual inoculation suggested competition between micro-organisms (31).

In yet another study conducted at the above place, two acid producing bacteria, (*Agrobacterium radiobacter* and *Agrobacterium* sp.), and/or *Laccaria laccata* were included in the rhizosphere of beech and pine seedlings grown on sand mixed with rock phosphate and phlogopite (iron-magnesium

mica). After two years, *A. radiobacter* or *L. laccata* root inoculation increased growth and nutrient uptake (P, Ca, K, and Mg) in beech but dual inoculation had no significant effect on plant growth and nutrient uptake. With pine seedlings, the growth and nutrient uptake promoting effect was observed only in inoculation with *Agrobacterium* sp. and only in the root system. Bacterial inoculation increased the amount of organic acids (malic acid, lactic acid, fumaric acid, and citric acid) released on the rhizosphere which promoted mineral element solubilization and the plant mineral nutrition. Mycorrhizal plants did not release greater amounts of organic acids than non-mycorrhizal plants (32).

On agricultural crops

Studies conducted at the Ruakara Soil and Plant Research Station, Hamilton, New Zealand, showed that inoculation of tomato (*Lycopersicon esculentum* Mill. Virosa NT2) plants in pot cultures with phosphate rock dissolving bacteria (PRDB) alone or in combination with *Gigaspora margarita* or *Gigaspora margarita* + sulphur oxidizing Thiobacilli did not affect shoot dry matter production or phosphorus uptake. Inoculation with PRDB in initial stages and with thiobacilli had a detrimental effect on shoot dry matter production. The PRDB population declined soon after soil inoculation but recovered after the addition of phosphate-free nutrient solution. It is suggested that soil bacteriostasis and a deficiency of easily metabolized carbon compounds were the cause of failure of the PRDB culture (28). Studies conducted at the Institute Zootecnia Caixa, Nova Odessa, Brazil, showed that *Centrosema pubescens* plants inoculated with *Glomus fasciculatum* showed more efficient nutrient assimilation, biomass production, nodulation, and nitrogenase activity in a medium amended with two rock phosphates and these effects were enhanced in the presence of phosphorus solubilizing organisms: a fungus and a bacterium (46). In pot experiments conducted at the Indian Agricultural Research Institute, New Delhi, India, on lentils cv. L-5-9 (*Lens esculenta*), highest mean grain yield, mean hay yields, and mean phosphorus uptake (3.20 g/pot, 6.74 g/pot and 48.30 mg P_2O_5/pot respectively) were obtained after inoculation with both *Glomus fasciculatum* and *Pseudomonas striata*. The next highest were obtained after inoculation with *Aspergillus awamori* alone. Of all the fertilizer treatments, application of 45 kg of P_2O_5 per ha each of rock phosphate and superphosphate with farmyard manure produced the highest mean grain yields, mean hay yield and phosphorus uptake of 3.49 g/pot, 9.13 g/pot, and 59.46 mg P_2O_5/pot respectively (51). In another study conducted at the above Institute, inoculation of wheat with *Pseudomonas striata, Agrobacterium radiobacter*, and VAM fungi (*Glomus fasciculatum*, and *Gigaspora margarita*) improved dry matter yield but nitrogen and phosphorus fertilizers significantly gave better yield. Maximum yield was, however, obtained with microbial inoculation and fertilizers together (21). In studies conducted at the University of Agricultural Sciences, Bangalore, India, dual inoculation with *Glomus fasciculatum* and one of the two phosphate solubilizing fungi, *Penicillium funiculosum* or *Aspergillus niger* produced a synergistic interaction and resulted in improved growth and nutrient uptake in *Setaria italica* (22).

Studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad, Karnataka, India, showed that combined inoculation of chilli with *Glomus macrocarpum* and *Bacillus polymyxa* was better than individual inoculation and produced highest fruit yield. It was possible to replace 25% of the phosphorus with combined inoculation as compared to individual inoculation (13).

Studies conducted at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India, on *Pennisetum padicillatum* showed that seed inoculation with PSB and nitrogen fixing bacteria (NFB) resulted in increases in root volume, VAM colonization, and VAM spore number in the presence of *Glomus macrocarpum*. Such stimulation was more with NFBs than with PSBs (57). Studies conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore, India, on neem (*Azadirachta indica*) showed that combined inoculation of neem plants with VAM fungi and phosphobacterium (*Pseudomonas striata*) enhanced the dry matter production, VAM colonization, and plant nutrient uptake significantly as compared to individual inoculation and uninoculated controls (25). Studies conducted at the Estacion Experimental del Zaidin, CSIC, Granada, Spain, on nodulated *Pueraria phaseoloides* showed that the plant-Rhizobium (soil inoculation)–mycorrhiza symbiosis system may result in an increase in the yield and nutrition in association with specific rhizosphere bacteria that solubilize rock phosphate (calcium phosphate) (four isolates), iron phosphate (two isolates), and aluminium phosphate (two isolates). Considerable stimulation of uptake of nutrients (nitrogen, phosphorus, potassium, and calcium) was observed with VAM-bacteria combinations of *Azospirillum* sp. (1), *Bacillus* sp. (1) or *Enterobacter* sp. (1 or 2) associated with *Glomus mosseae*. However, *Bacillus* sp. (1), a calcium phosphate solubilizing isolate, positively interacted with *G. mosseae* and negatively with *G.*

fasciculatum. This shows that there is a specific functional compatibility between the biotic components integrated in the system.

Studies also showed that iron phosphate solubilizing micro-organisms were more active alone than in dual association with *Glomus* sp. but the aluminium phosphate dissolving isolates positively interacted with mycorrhizal plants (61). Further studies at the above Institute on *Glycine max* inoculated with *Glomus mosseae* or *Glomus* sp. and *Rhizobium japonicum* in a neutral calcareous soil showed that phosphorus solubilizing bacteria did not improve phosphorus utilization either by roots or mycorrhizae but increased shoot nitrogen concentration and content, shoot to root ratio, and amount of mycorrhizal infection at all levels of added tricalcium phosphate (7).

Interaction between actinomycetes and mycorrhiza

Studies conducted at the Tel Aviv University, Israel, on actinomycetes isolated from well-developed forests and from an adjacent clear-cut area with impaired *Pseudotsuga menziesii* regeneration for two decades showed that isolates from clear-cut areas showed five times the phytotoxic effect of those from forest areas. Some actinomycetes isolates, four per cent from clear-cut area and 2.6% from forest area, significantly reduced *in vitro* growth of two common ectomycorrhizal fungi of *P. menziesii, Laccaria laccata* and *Hebeloma crustuliniforme*. Out of these, two actinomycetes isolates from clear-cut area reduced fungal growth by 40% and 73% respectively. Reduction of nutrients in the growth medium did not affect the antifungal activity of actinomycetes (17).

Studies conducted at the Copernicus University, Torun, Poland, also showed that actinomycetes isolated from rhizosphere and non-rhizosphere of Scots pine (*Pinus sylvestris*) seedlings growing in unacidified or artificially acidified soils inhibited *in vitro* growth of ectomycorrhizal fungi. The inhibition was more pronounced for *Rhizopogon vinicolor* and *Hebeloma crustuliniforme* than *Laccaria laccata*. Actinomycetes from unacidified soil were more effective than from acidified pine soil. There was, however, no difference in actinomycetes isolated from rhizosphere and non-rhizosphere soil in their effect on fungal growth but the effect on fungal growth was dependent on the medium used (50).

Studies conducted at the USDA-ARS Western Regional Research Centre, Albany, USA, showed that seven out of twelve actinomycetes (eight *Streptomyces*; two *Nocordia*, and two unidentified) isolated from spores of *Glomus macrocarpum* and inoculated into unsterilized soil containing *G. macrocarpum* and *G. mosseae* significantly increased the per cent mycorrhizal root colonization and four significantly increased the density of hyphae 5 μm in diameter, predominantly those of VAM fungi in roots of onion plants. None of the actinomycetes adversely affected the plant growth or nutrient content though chitin decomposing actinomycetes were strongly antagonistic to fungi, bacteria or actinomycetes or combination of these (2).

Studies conducted at the School of Forestry and Wood Products, Michigan Technological University, Houghton, USA, showed that most of the actinomycetes isolated from mycorrhizoplane of red pine (*Pinus resinosa*) seedlings recently outplanted at the cleared northern broad leaf sites exerted a range of effects on the growth of three ectomycorrhizal fungi namely *Laccaria bicolor, L. laccata,* and *Thelephora toorestris* in *in vitro* studies during the four-week test period. Some actinomycetes inhibited while others stimulated the growth of ectomycorrhizal fungi (49).

Studies conducted at the Department of Agronomy, Iowa State University, Ames, USA, showed that some actinomycetes isolated from the soil enhanced spore germination of *Gigaspora margarita* whereas others did not. When placed in a common head space with actinomycetes but physically separated in a divided petri dish, the spores showed germination rates of up to 73% as compared to controls (without actinomycetes) which showed 23% spore germination after 11 days. Also, actinomycetes having straight spore bearing hyphae stimulated VAM spore germination more than actinomycetes having spiral spore bearing hyphae (11).

Soil microflora associated with mycorrhizal fungal elements

Studies conducted at the Commonwealth Scientific and Industrial Organization (CSIRO), Glen, Osmand, Australia, showed that the majority of the organisms isolated from inside the mantle of ectomycorrhizae formed by *Rhizopogon luteolus* on the roots of *Pinus radiata* growing on a sandy podzol had a stimulating effect on mycelial growth of *R. luteolus* and/or mycorrhiza formation. A significant microbial population was present within the mantles of ectomycorrhizae; gram negative bacteria being the dominant among them (19). Studies conducted at the University of Toronto, Canada, showed that most common microfungi isolated from serially washed ectomycorrhizae of *Picea mariana* included *Mycelium radices-atrovirens* alpha, an undescribed non-sporulating fungus, *Micromucor isabellinus, Penicillium spinulosum*, and *P. montanense*. None

of these fungi had a high degree of specificity for the mycorrhizal mantles. These are relatively similar to those obtained from suberized root surfaces and from assimilative rootlets of *Cornus canadensis* (59).

Studies conducted at the INRA, Centre de Recherché Forestieres de Nancy Champenoux, Seichamps, France, on bacteria isolated from mycorrhizae and sporocarps of *Laccaria laccata* showed that some bacteria reduced infection of roots of *Pseudotsuga menziesii* by *L. laccata* while others stimulated the infection both in the greenhouse and in the bare root nursery (20). In studies conducted at the Czechoslovak Academy of Sciences, Prague, Czechoslovakia, all the bacteria isolated from the hyphosphere of extramatrical mycelium of *Glomus fasciculatum* growing in symbiosis with roots of *Trifolium repens, Hibiscus elatus*, and *Exonopus gracilis* were gram negative rods but no fluorescent Pseudomonads were present. Most of those present required aminoacids (tyrosine, aspartic acid, and ornithine) and growth factors. The results show that VAM fungi select hyphosphere bacteria previously selected by the plant (62). In studies conducted at the USDA-ARS Western Regional Research Centre, Albany, California, USA, spores of *Glomus macrocarpum* isolated from a calcareous, silty clay loam soil were plated on chitin agar for isolation of actimycetes. Out of 190 spores, 100 were colonized by one or more chitin decomposing micro-organisms. Out of these 100 spores, 82 were colonized by actinomycetes, 17 by bacteria, and 1 by fungi. Out of 51 identified actinomycetes, 29 belonged to *Streptomyces*, 3 to *Nocardia*, 1 to *Streptosporangium*, 1 to *Nocardioides*, and 15 were unidentified (3). Studies conducted at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India, showed that several bacteria isolated from sporocarps of mushrooms could be used as biofertilizers to produce VAM inocula blended with the bacteria to boost up the plant growth (44).

Parasitic soil microflora on mycorrhizae/mycorrhizal fungi

In studies conducted at Tubingen University, Germany, intracellular infection of mycorrhizae of *Picea abies* was found in the vascular tissues by *Cryptosporiopsis abietina* and in the meristem by *Mycelium radices-atrovirens*. Affected hyphal mantles and cortex were killed resulting in hollow spaces surrounded by cells filled by tannins in the vascular tissues. Above ground declining symptoms were also thought to be connected with malnutrition due to dying of mycorrhiza (23). Studies conducted at the University of Punjab, Lahore, Pakistan, showed that many endogonaceous spores isolated from the soil were found to be colonized by unspecified fungi and bacteria causing various degrees of disintegration (40). In another study conducted at the above place, *Thermomyces stellatus* suppressed hyphal growth and vigour of *Glomus* sp. and *Sclerocystis pakistanica* on samples of scale leaves of *Chlorophyton*. Mycelium, vesicles, and spores of VAM fungi shrivelled when in contact with the hyphae of *T. stellatus* (39).

Studies conducted at the University College, Dublin, Irish Republic, showed frequent emergence of dark, sterile mycelia and *Oidiodendron* spp., predominantly *O. maius*, when segments of excised tips of mycorrhizal roots from *Picea sitchensis* were plated after surface sterilization. These fungi limited the isolation of the potential mycorrhizal symbionts. Pure culture synthesis confirmed the ectomycorrhizal ability of potential symbiont isolates (obtained after the addition of benomyl to the culture medium) and demonstrated the parasitic nature of dark, sterile mycelial types (52).

In studies conducted at the University of Rhode Islands, Department of Botany, Kingston, USA, 272 isolates were cultured from crushed *Gigaspora gigantea* spores, collected from a maritime sand dune in four different stages of vigour (newly formed spores; greenish yellow, healthy spores; yellow, moribund, mottled spores with brown spots; brown, dead, blackened, and collapsed spores). Of these, 44 species of fungi and 6 actinomycetes were identified. Most frequently occurring fungi were *Acremonium* sp., *Chrysosporium parvum, Exophiala wermeckii, Trichoderma* sp., and *Verticillium* sp. Twenty-one of the isolated species could function as pathogens forming either internal projections as in *Acremonium* sp., *Chrysosporium parvum, Cladosporium* sp., *Geomyces pannorum, Oidiodendron* sp., *Sporothrix* sp. *Verticillium* sp., and actinomycetes *Nocardia* sp. or fine radial canals or both. The internal projection length was positively correlated with duration of exposure to the parasites. Both internal projections and fine radial canals were formed in live spores while the heat-killed spores possessed fine radial canals only. Pathogenicity differed among the parasites and was greatest for *Verticillum* and *Acremonium* (29).

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Research findings · · ·

Under this column appear short notes on important breakthroughs/significant achievements in original research of high calibre in the field of mycorrhizae, which have not yet been published.

Effect of single and multiple VAM inoculants on the growth parameters of tomato

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Arbuscular mycorrhiza benefits the plant by improving the supply of nutrients, especially phosphorus and other minerals such as, Zn, Cu, S, K, and Ca (Cooper, Tinker 1978) and the plant supplies the fungus with photosynthetic sugars (Verma, Schuepp 1995). Pure cultures of single species of vesicular–arbuscular mycorrhizal (VAM) fungus are being assessed for the appreciable plant growth and crop production. VAM fungi increased biomass production of sustainable agricultural crops. Multiple inoculation of the plants with VAM fungi has often yielded increased biomass production, but less emphasis has been paid to exploit their practical

utilization (Hepper, Azcon, Rosendahl, Sen 1987; Sieverding 1988; Kumar 1990). In the present study, three species of VAM fungi, i.e., *Glomus constrictum* Trappe, *Glomus mosseae* (Nicolson, Gerdemann) Gerdemann and Trappe, and *Glomus fasciculatum* (Thaxter Sensu Gerd) Gerdemann and Trappe have been assessed, singly and in combination, for their efficacy as potential VAM inoculants for tomato.

Materials and method

Three species of VAM fungi were screened for their efficacy. Pure culture of all the three VAM species which were maintained on buffel grass (*Cenchrus cillaris* L.) in the 12 inches pots containing sands were used for inoculation. Steam-sterilized sandy loam soil sand mix (1:1 volume/volume) substrate was dried and potted in 1 kg capacity clay pots. Sixty grams of single or mixed dry soil inoculum containing 400–450 spores/50 g soil was mixed in the top 6 cm of the soil of each treatment pot. Control plants received 60 g of soil containing non-mycorrhizal root pieces of buffel grass. Preliminary studies with the standardization of the dose of soil inoculum showed that this quantity of mycorrhizal inoculum was sufficient for the production of a reasonable amount of infection. The amount of VAM inoculum for each treatment was so adjusted that equal quantity of soil inoculum could be added to each pot. The different doses of soil inoculum used for each treatment were as follows:

- single endophyte—60 g,
- double endophyte—30 + 30 g, and
- triple endophyte—20 + 20 + 20 g.

Each treatment was replicated seven times, and the following treatments were included in the study:

- soil without inoculation (control),
- soil inoculated with *G. constrictum* (60 g),
- soil inoculated with *G. mosseae* (60 g),
- soil inoculated with *G. fasciculatum* (60 g),
- soil inoculated with *G. constrictum* (30 g) and inoculum of *G. mosseae* (30 g),
- soil inoculated with *G. constrictum* (30 g) and inoculum of *G. fasciculatum*,
- soil inoculated with *G. mosseae* (30 g) and inoculum of *G. fasciculatum* (30 g), and
- soil inoculated with *G. constrictum* (20 g) *G. mosseae* (20 g) and inoculum of *G. fasciculatum* (20 g).

Five surface-sterilized (10% sodium hypochlorite for three minutes) seeds of tomato (*Lycopersicon esculentum* L. cv. Pusa Ruby) were sown in each pot above the soil inoculum. Seedlings were thinned to one seedling/pot seven days after germination, pots were maintained under greenhouse conditions. Plants were uprooted periodically and per cent colonization of the roots was assessed by methods of Phillips, Hayman (1970). The number of VAM fungal spores were extracted from the rhizosphere soil by wet sieving and decanting method (Gerdemann, Nicolson 1963) and spore count was recorded. Biomass production was recorded in the term of shoot and root fresh weight and shoot and root dry weight, phosphorus content in the shoots and roots in term of g/mg tissue (Anderson, Ingram 1989). The data were statistically analysed.

Results

All the three VAM fungi were tested for their efficacy on tomato singly and in various combinations. All the treatments showed improvement in the mycorrhizal colonization, number of chlamydospores recovered from the rhizosphere soil, plant biomass, and P contents of the shoots and roots. However, all the three VAM fungi tested behave differently in different combinations and differ from one species to another species. Maximum growth in plants and spore count was observed by the inoculation of *G. mosseae* followed by *G. constrictum* and *G. fasciculatum* respectively. In combination the maximum increase was observed in the plants which received *G. mosseae* and *G. constrictum* followed by *G. constrictum* and *G. fasciculatum* and *G. mosseae* and *G. fasciculatum* respectively. However, highest increase was observed in the shoot biomass, mycorrhizal colonization in P content in the shoots and roots when the plants were inoculated with all the three VAM fungi (Table 1).

These enhancements in the plant growth parameters, mycorrhizal colonization, and biomass production may be reflected in higher yield of tomato due to the multiple inoculation of VAM and this deserves due attention and further emphasis to confirm the suitability of multiple inoculum as compared to single inoculum for better crop production. Mycorrhizal symbiosis is an attractive process in agriculture and forest management to enhance crop and wood production in the sense of sustainable agriculture and restoring soil fertility (Verma 1995).

Many attempts have been made, over the years, to determine the influence of mixed VAM fungal species on root colonization and sporulation. There are numerous reports of increased plant growth from inoculation with non-indigenous VAM species even into non-sterile soil containing indigenous species. The competitive ability of *Glomus tenue* was dem-

Table 1. Effect of single and multiple inocula of VAM on the growth of tomato, cv Pusa Ruby in sterilized soil under pot conditions

Treatment	Shoot fresh wt (g)	Root fresh wt (g)	Shoot dry wt (g)	Root dry wt (g)	Phosphorus µg/mg		Colonization (%)	No. of chlamydospores recovered from 20 g soil
					Shoot	Root		
Control	39.20	3.2	3.32	0.20	1.05	0.25	10	32
G. constrictum (Gc)	62.5	5.0	5.85	0.68	1.23	0.17	86	170
G. mosseae (Gm)	53.3	4.9	4.85	0.62	1.12	0.30	80	196
G. fasciculatum (Gf)	41.5	4.6	3.82	0.31	1.10	0.27	75	141
Gc + Gm	69.4	9.5	9.42	1.63	1.70	0.69	67	117
Gc + Gf	65.5	8.0	8.00	1.27	1.62	0.55	70	115
Gm + Gf	63.0	6.2	6.75	0.90	1.45	0.34	61	102
Gc + Gm + Gf	74.3	10.8	12.00	2.00	1.95	0.90	90	95

Note: Each value is the mean of five replicates and significant at five per cent.

onstrated by Powell, Daniel (1978), who observed an increase in plant growth when *Glomus tenue* was added to host pot cultures already colonized by other VAM fungi. Further evidence of competition between VAM fungal species came from Ross, Ruttencutter (1977) who found that less colonization occurred in *Glomus macrocarpum* inoculated peanuts and soyabean as compared to hosts inoculated with *Gigaspora gigantea* and *Glomus macrocarpum* together.

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Studies on VAM association in Marwar Teak [*Tecomella undulata* (Smith) Seeman] in desert soils of Rajasthan

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Many plant species are commonly grown in the sand-dune and desert soils of Rajasthan. They are important sources of tannin, gum, fodder, fuel, and timber. Among them, *Tecomella undulata* (Smith) Seeman, a member of the family Bignoniaceae is one of the co-dominant tree species in the desert region of western Rajasthan and adjoining areas of Haryana. It is commonly known as Marwar Teak and Rohida. This tree species thrives very well on stabilized sand-dunes and tolerates extremes of temperatures in summer and winter. It acts as a soil binding tree by spreading a network of lateral roots on the top surface of the soil and thus plays an important role in environmental conservation. This is economically important and a valuable timber tree species of Rajasthan. The wood is used for good quality furniture, doors, windows, agricultural implements, and toys. Leaves of the tree are eaten by cattle and goats. Flowers and pods are consumed by camels, goats, and sheep. The trees are evergreen to deciduous, bloom in winter (December–February), the capsules start ripening in April and are fully ripe in May. Also, it is an agro-forestry species for arid climates and a large population is found in agricultural lands in association with *Prosopis cineraria* and *Acacia nilotica*. Information on the status of VAM fungi in association with this tree species is lacking. Considering the importance of the tree species, the present investigation was carried out to study the type of mycorrhizal associations and scan out the VAM fungal population from the rhizosphere of *T. undulata* in the arid zone of Rajasthan.

Materials and method

Surveys were conducted to collect root and rhizosphere soil samples of *T. undulata* from different Forest Department nurseries and plantations located in and around Jodhpur district of Rajasthan. Initially, attempts were made to detect the type of mycorrhizal association by examining root sections and cleared root samples by adopting the method given by Phillips, Hayman (1970). Per cent root colonization was determined based on the number of root segments colonized by VAM fungi. VAM fungal propagules were isolated from the rhizosphere soils collected from nurseries and plantations by using wet sieving and decanting technique (Gerdemann, Nicolson 1963). Number of VAM propagules present in the soil were calculated to 100 gm dry weight of the soil. The species level identification of different fungi was done following the keys provided by Trappe (1982) and Schenck, Perex (1987).

Results and discussion

Mycorrhizal status in roots and rhizosphere of T. undulata *in nursery and plantation sites*

Results on per cent root colonization and soil spore population of VAM fungi in different nursery and plantation samples of *T. undulata* are shown in Table 1. It revealed that both nursery and plantation samples had extent of colonization in roots and spores in soil but variation in distribution. More colonization (per cent) and spore number of VAM fungi were recorded in plantations than in nursery samples. It was observed that maximum per cent colonization and VAM spores were found in the samples collected from Osian (64% and 184) and Phalodi (60% and 163) nurseries. The least number of spores and per cent colonization was recorded in the samples collected from Lokswell nursery. Similarly, maximum colonization and num-

Table 1. VAM fungal status of *T. undulata* in nursery and plantation sites in arid zone of Rajasthan

Sites	Root colonization (%)	Soil spore population (spores/100 g soil)
Nursery sites		
Jhalamand	54.5	142
Lokswell	38.3	98
Osian	64.4	184
Phalodi	60.0	163
Tinwari	46.8	123
Standard error (m)	3.16	8.79
CD at five per cent level	7.71	21.45
Plantation sites		
Bapupura	56.0	104
Chaukha	66.0	204
Chopasni	89.0	252
Gudavoisnivion	93.0	328
Jhalamand	50.0	109
Mogera	83.3	218
Osian	62.0	192
Standard error (m)	2.99	8.12
CD at five per cent level	7.17	19.48

Table 2. Frequency distribution of VAM fungi in nursery and plantation sites of *T. undulata* in arid zone of Rajasthan

VAM fungi	Nursery sites					Site frequency	Plantation sites							Site frequency
	1	2	3	4	5	(%)	1	2	3	4	5	6	7	(%)
Glomus clarum	–	–	+	–	–	20	–	–	–	–	–	–	–	—
G. claroideum	–	–	–	–	–	—	–	–	–	–	+	+	–	29
G. fasciculatum	+	+	+	+	–	80	+	+	+	–	+	+	+	86
G. fulvus	–	–	–	–	–	—	–	+	–	–	–	–	–	14
G. intraradices	–	–	–	–	–	—	–	+	–	–	–	+	–	29
G. macrocarpum	–	–	–	–	+	20	–	–	+	–	–	–	–	14
G. microcarpum	+	+	+	+	+	100	+	–	+	–	+	–	+	57
G. monosporum	–	–	–	–	–	—	–	+	–	–	+	–	–	29
G. occultum	–	+	+	+	–	60	+	+	–	+	+	–	+	71
G. pubescens	–	–	–	–	–	—	+	–	–	–	+	+	–	43
Gigaspora sp.	–	–	–	–	–	—	–	–	–	+	–	+	+	43
Sclerocystis sp.	–	–	+	+	–	40	–	–	–	–	–	–	–	—

ber of spores were recorded in the plantation samples collected from Gudavoisnivion (93% and 328) followed by Chopasni (89% and 252), and Mogera (83% and 218). Least colonization and spore number were observed in the samples collected from Jhalamand and Bapupura.

Frequency distribution of mycorrhizal fungi in nursery and plantation samples of T. undulata

The frequency distribution of different VAM fungi isolated from the rhizosphere of *T. undulata* plants in nursery and plantation sites is shown in Table 2. It was found that almost all the soil samples contained VAM spores of different species of the genera viz. *Glomus, Gigaspora*, and *Sclerocystis*. A total of six different species of two genera such as *Glomus* and *Sclerocystis* were isolated and identified from the rhizosphere of nursery samples. Among the different species of the genus *Glomus, G. microcarpum* was found to be the most frequent fungus (100%) followed by *G. fasciculatum* (80%) and *G. occultum* (60%).

A total of 10 different VAM fungal species belonging to two genera viz., *Glomus* and *Gigaspora* were isolated and identified from the rhizosphere of plantation samples. Among the species of the genus *Glomus, G. fasciculatum* was found to be the most frequent fungus (86%) followed by *G. occultum* (71%) and *G. microcarpum* (56%). The genus *Glomus* was found to be the most predominant both in nursery and plantation samples. The genera *Sclerocystis* and *Gigaspora* were represented by a single species only in nursery and plantation samples respectively.

The study corroborates with the earlier findings of VAM association with tree species in different parts of the country by many researchers (Thapar, Khan 1973, 1985, 1988; Mukerji, Kapoor 1986; Tarafdar, Rao 1990; Thapar, Vijayan, Uniyal 1992; Mohan, Verma, Singh 1995; Mohan, Singh 1996, 1997). Earlier studies by the authors have shown that variation in per cent colonization in roots and VAM spores in the rhizosphere soils of various arid zone tree species under different site/soil conditions (Mohan, Verma 1995; Mohan, Verma, Singh 1995; Mohan, Singh 1996, 1997). The intensity of VAM colonization in roots and spores in the rhizosphere of *T. undulata* in nursery and plantation sites varied according to the age of the plants and site factors including soil nutrients, moisture, and other environmental conditions.

Glomus fasciculatum, G. microcarpum, and *G. occultum* are widespread and consistent, both in nurseries and plantations, which makes them the most favourable for mass multiplication as well as seedling inoculations of *T. undulata* in the arid zone/desert ecosystem of Rajasthan.

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New approaches . . .

Under this column appear brief accounts of new techniques, modifications of available techniques, and new applications of other known techniques, etc., in mycorrhiza research that have been published in reputed journals during the last two or three years.

Root organ culture: a tool for molecular studies on VAM fungi

Root organ culture (ROC) is a true representative system of soil-grown roots as it mimics the essential features of the complex rhizosphere, allowing the fungi to complete their life-cycle in the presence of host roots without any induced genetic changes. Gadkar V, Adholeya A, Satyanarayna T (1997) used randomly amplified polymorphic DNA with the M13 mini-satellite sequence as the polymerase chain reaction primer to look into polymorphism, if any, in the spores of *Gigaspora margarita* produced by ROC system and *in situ* (soil) (*Canadian Journal of Microbiology* **43**: 795–798). The authors found in their studies that fingerprint pattern of soil-grown spores (non-germinated) was similar to that of the mother spore (germinated) that was exposed to the synthetic growth conditions of ROC, indicating that the defined conditions of ROC do not cause a change in the spore genotype even after germination. Also, the fingerprint pattern of auxiliary cell cluster demonstrated genetic similarity between extramatrical structure and the spore. The authors also found that the mother spore which seemed hollow and inactive four months after germination under ROC system contained nuclei and generated a fingerprint pattern. The authors also found that the fingerprint pattern of *Gigaspora margarita* was different from that of *G. gigantea* indicating that the mini-satellite sequence could be exploited for identifying VAM fungi. It is thus evident that the ROC system can be used as a tool for conducting varied types of molecular studies such as molecular taxonomy, genetical studies, etc. on VA mycorrhizal fungi.

Forthcoming events · · ·

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13–17 August 1998
16th International Conference on Plant Growth Substances. Makuhari Messe, Chiba, Japan.
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15–20 August 1998
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News from members · · ·

Prof. Bushan L Jalali, Department of Plant Pathology, Haryana Agricultural University, has been elected President of the Indian Society of Mycology and Plant Pathology. This premier national society has been directing its efforts for the sustainable management of crop diseases at the national level. Prof. Jalali has made notable contributions, spanning over two decades, in the areas of integrated plant disease management using innovative mycorrhizal technology.

Dr I L Kothari has been elected Head of Biosciences Department and Co-ordinator of Special Assistance Programme of University Grants Commission (UGC) on Plant Morphogenesis at Sardar Patel University, Vallabh Vidyanagar, Gujarat, India. His research paper entitled *Mycorrhizal Biodiversity of Petro-effluent Irrigated Fields* (I L Kothari, Udhaya Joseph, and C R Patel) has been published in the *Journal of Mycology and Plant Pathology* **27**(3): 275–278, 1997. He has also organized the *UGC National Symposium-cum-workshop on Plant Structure and Morphogenesis* at the Sardar Patel University on 27–28 December 1997. A proceeding volume commemorating contributions of Prof. Y S Dave was released on this occasion.

Recent references · · ·

Latest additions to the Network's database on mycorrhiza are published here for information of the members. The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge.

This list consists of papers from the following journals, which are arranged in alphabetical order.

- *Applied Soil Ecology*
- *Canadian Journal of Botany*
- *Canadian Journal of Forest Research*
- *Economic Botany*
- *Environmental Pollution*
- *FEMS Microbiology Letters*
- *Journal of Agricultural Science*
- *Journal of Environmental Quality*
- *Journal of General and Applied Microbiology*
- *Journal of Horticultural Science & Biotechnology*
- *Journal of the Japanese Society for Horticultural Science*
- *Journal of Mycology and Plant Pathology*
- *Mycologia*
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- *Plant and Soil*
- *Restoration Ecology*
- *South African Journal of Botany*
- *Water Air and Soil Pollution*

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

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S No.	Germplasm bank code	Fungus name	Host	Source person/place of origin
Ectomycorrhizae				
1.	EM-1025	*Rhizopogon vulgaris*	*Pinus Ponderosa, Pseudotsuga menziesii,* Abies	Oregon, USA
2.	EM-1271	*Pisolithus tinctorius*	*Pinus elliotii*	Georgia, USA
3.	EM-1055	*Phaeolepiota aurea*	Sitka spruce	Alaska, USA
4.	EM-1134	*Paxillus involutus*	*Picea pungens,* Betula	Oregon, USA
5.	EM-1130	*Melanogaster tuberiformis*	*Aretostaphylos viscidia*	—
6.	EM-1264	*Lactarius rufus*	*Sous resineux*	Sweden
7.	EM-1195	*Laccaria proxima*	—	Czechoslovakia
8.	EM-1193	*Laccaria laccata*	Sitka spruce	Cumbria
9.	EM-1188	*Laccaria laccata*	*Picea sitchensis*	Edinburgh, UK
10.	EM-1187	*Laccaria bicolor*	—	Hammarskog
11.	EM-1185	*Hysterangium incarceratum*	Eucalyptus sp.	—
12.	EM-1180	*Hebeloma sinapizans*	Picea	Lyon, France
13.	EM-1170	*Hebeloma crustuliniforme*	Hetre	Vosges, France

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