About TERI
A dynamic and flexible organization with a global vision and a local focus, TERI (The Energy and Resources Institute, formerly known as the Tata Energy Research Institute) was established in 1974. TERI’s focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lie in TERI’s firm belief that the efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

Biotechnology and Management of Bioresources Division
Focusing on ecological, environmental, and food security issues, the activities of the Biotechnology and Management of Bioresources Division include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grass-roots level, with village communities.

Mycorrhiza Network
The Biotechnology and Management of Bioresources Division’s Mycorrhiza Network works through its three wings—the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhizal Culture Collection), and Mycorrhiza News.

The MIC is primarily responsible for establishing databases on Asian mycorrhizologists and on mycorrhizal literature (RIZA), which allows information retrieval and supplies documents on request. The CMCC has the main objectives of procuring strains of both ecto and vesicular arbuscular mycorrhizal fungi from India and abroad; multiplying and maintaining these fungi in pure cultures; screening, isolating, identifying, multiplying, and maintaining native mycorrhizal fungi; developing a database on cultures maintained; and providing starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.

Mycorrhiza News
Mycorrhiza News – a quarterly newsletter publishing articles compiled from RIZA – invites short papers from budding and senior mycorrhizologists, giving methodologies being used in mycorrhizal research, providing information on forthcoming events on mycorrhiza and related subjects, listing important research references published during the quarter, and highlighting the activities of the CMCC.
Condolence

With profound grief, we inform the passing away of Dr Sujan Singh, at his home on 9 December 2007. He was the Coordinator of the Mycorrhiza Network, TERI, since its inception in 1987. We pay tribute to his invaluable contribution to its growth and deeply mourn the loss.

Contents

Effect of mycorrhiza on production of antifungal compounds 3

Research finding papers
Phosphatase activity in the rhizosphere of medicinal plants inoculated with arbuscular mycorrhizal fungi 11
Response of Rangpur lime to different endomycorrhizal species for germination, growth parameters, and graft success 13
Vesicular arbuscular mycorrhizal association in Bryophytes isolated from Eastern and Western Himalayas 16

Studies on arbuscular mycorrhizal fungi of Maranta arundinacea L. and associated plants in different soils of Kanyakumari district 20

New approaches
A quick and precise technique for identifying ectomycorrhizas by PCR 23

Centre for Mycorrhizal Culture Collection
Effect of entomopathogenic nematode on arbuscular mycorrhizal fungi inoculated tomato plant’s growth, mycorrhizal colonization, and enzyme activity of hyphae 24
Recent references 27
Forthcoming events 31
Effect of mycorrhiza on production of antifungal compounds

Sujan Singh
TERI, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi – 110 003, India

Phenolic compounds impart resistance to plants against pathogenic organisms. Mycorrhiza infection in plant roots results in enhancement of phenolic contents in plants.

Effect of vesicular arbuscular mycorrhizal infection on phenolic compounds

Studies conducted at the Department of Botany, AVVM Sri Pushpam College, Poondi, Tamil Nadu, India, showed that mycorrhizal inoculation of Sesamum indicum roots resulted in a significant enhancement of the percentage of infection, dry matter, and phenolic content of plants in sterilized soils compared to non-inoculated plants. Histological studies revealed an accumulation of different types of lipids and phenolic compounds in VAM (vesicular arbuscular mycorrhizal) structures, particularly neutral lipids and catechol tannins in vesicles. Such increased amounts of lipids and phenols in mycorrhizal plants could be the contribution of fungal structures (Selvaraj and Subramanian 1990).

In studies conducted at the Station de Recherches, Agriculture Canada, total free phenols extracted from 10 kg of fresh field-grown onion roots were separated by preparative HPLC (high performance liquid chromatography), using an acidic methanol gradient on a Nova-Pack C18 (4 µm) reverse phase column. Major peaks were simultaneously detected at 340 nm and 254 nm. Those exhibiting homogeneity greater than 70% were further purified on the same column using an appropriate isocratic mode, run with aqueous acidic acetonitrile. Each molecular entity was then recrystallized prior to NMR (nuclear magnetic resonance) – proton and carbon– analysis followed by MS (mass spectra) determination. Among the common cinnamic derivatives, two cinnamoyl amides were detected in important quantities. Chemical synthesis of cinnamic derivatives, two cinnamoyl amides were detected in important quantities. Chemical synthesis of cinnamic derivatives, two cinnamoyl amides were detected in important quantities. Chemical synthesis of cinnamic derivatives, two cinnamoyl amides were detected in important quantities. Chemical synthesis of cinnamic derivatives, two cinnamoyl amides were detected in important quantities.
not found in carrot roots (Nagahashi, Abney, and Doner 1996).

**Effect of ectomycorrhizal infection on phenolic compounds**

In studies conducted at the Institut für Botanik, Spezielle Botanik, Mkyologie, Eberhard-Karls-Universität, Tübingen, Germany, p-H BA glucoside, picein, piceatannol and its glucoside,isorhamptonin, catechin, and ferulic acid could be identified by HPLC in mycorrhizas of *Picea abies* – *Lactarius deterrimus* and *P. abies* – *Laccaria amethystina*, collected from axenic cultures (the latter was also collected from a spruce stand). The same phenolics occurred in non-mycorrhizal short roots from sterile cultures. Amounts of p-H BA glucoside, picein, catechin, and cell wall-bound ferulic acid were considerably lower in mycorrhizas from axenic cultures, whereas those of the other phenolics were not significantly reduced. Pure mycelia of *L. amethystina* and *L. deterrimus* contained none of the identified phenolics (Münzenberger, Heilemann, et al. 1996).

Similar studies conducted at the Institut für Wald- und Forstokologie, D r. Zinn-Weg, 0-1300 Eberswalde, Germany, showed that ectomycorrhizas of *Larix decidua* (larch) – *L. amethystina* contained soluble phenolics, p-hydroxybenzoic acid glucoside, p-hydroxybenzoic glucose, picein, catechin and epicatechin. All compounds were strongly reduced in comparison to non-mycorrhizal short roots. The concentration of ferulic acid was also much lower in mycorrhizas than in non-mycorrhizal short roots. Ectomycorrhizas of *Larix decidua* contained phenolics in smaller quantities than ectomycorrhizas of spruce, a fact probably explaining the quicker mycorrhization of larch roots. In *ectendomycorrhizas* of *Arbutus unedo* – *L. amethystina*, the amount of phenolics was also much lower than in non-mycorrhizal roots. During mycorrhization of *P. abies*, *Larix* and *A. unedo*, no phenolic phytoalexins appeared. Pure mycelium of *Laccaria amethystina* contained none of the identified phenolics. The growth of the mycorrhizal fungus, *L. amethystina* and *L. deterrimus*, was strongly inhibited by most of the phenolic compounds. The reduction of phenolics, therefore, enabled the mycorrhizal fungus to establish the mycorrhizal state. This would indicate that the regulation by phenolics in the symbiotic ecto- and *ectendomycorrhiza* differs fundamentally from parasitic interactions (Münzenberger 1993; Münzenberger, Ottke, and Oberwinkler 1995).

In studies conducted at the Department of Botany, University of Hong Kong, Pokfulam Road, Hong Kong, Kottke, and Oberwinkler (1995). The cell walls of the outer cortex and the endodermis also contained suberin. Following an invasion by compatible ectomycorrhizal symbionts, condensed tannins disappeared from cells of the root cap and the epidermal layer, but hyphae were prevented from colonizing the cortex presumably due to suberin barriers. In vitro experiments indicated that a number of broad-host ectomycorrhizal fungi could utilise various polyphenolic compounds, including tannins found in the root-exudates of the host trees, with different degrees of efficiency (Tam and Griffiths 1993).

**Effect of VAM fungi on polyphenol oxidases and peroxidases**

In studies conducted at the Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India, the effect of the VAM fungus, *Glomus fasciculatum*, on isoenzyme patterns of greenhouse-grown *Ziziphus mauritiana*, *Ziziphus nummularia*, and *Ziziphus xylopyra* plants was investigated at 60 days after inoculation. Compared with untreated plants, all inoculated plants showed two additional peroxidase isoenzyme bands and an additional polyphenol oxidase band. In addition, both peroxidase and polyphenol oxidase showed increased activities (Mathur and Vyas 1995).

Furthermore, studies conducted at the above university on six VAM species namely – *Acaulospora morrowae*, *Gigaspora margarita*, *Glomus fasciculatum*, *Glomus macrocarpum*, *Scutellospora calospora*, and *Sclerocystis rubiformis* – collected from rhizosphere soils of *Ziziphus xylopyra* and evaluated under glasshouse conditions, showed that all fungi had beneficial effects on enhancement of various enzymes, proteins, phenolics, and catechin contents, with *S. calospora* having the greatest effect on all biochemical parameters. The enzymes included peroxidases and polyphenol oxidases among others (Mathur and Vyas 1996).

Studies conducted at the Pomology Department, Faculty of Agriculture, Alexandria University, Alex-Andria, Egypt, on one-year-old sour orange seedlings in black polyethylene bags containing a clay-loam soil with saline water (up to 4500 PPM [parts per million] salt [NaCl + CaCl2]) showed that salinity reduced peroxidase activity in leaves. The salinity, however, did not affect polyphenol oxidase (catechol oxidase) activity. Mycorrhizal infection increased polyphenol oxidase activity but did not affect that of peroxidase (Ezz and Nawar 1994).

Studies conducted at the Centro di Studio Sulla Micologia del Terreno, Dipartimento di Biologia Vegetale dell’Università di Torino, Viale M athio, Torino, Italy, on the activity of cell-wall-bound enzymes.
peroxidase in Allium porrum roots, measured during root growth and development of VA mycorrhiza with G. versiforme showed that the peak of activity in infected plants was higher than in non-infected ones and occurred during the initial stages of fungal penetration. When the infection was established and the roots were highly colonized, the activity decreased to the level of that in non-mycorrhizal roots. Utrastructural localization using diaminobenzidine revealed an increase in the electron dense deposits in the middle lamella corresponding with fungal penetration. No activity was seen in the matrix deposited around the intracellular fungal structures. The overall distribution of peroxidase activity was the same in mycorrhizal and non-mycorrhizal roots (Spami and Bonfante-Fasolo 1988).

Studies conducted at the CSIC, Department of Microbiology, Suelo and Sistemas Simbiot, Estacion Expt Zaidin, Granada, Spain, on examination of catalase and ascorbate peroxidase enzymatic activities during the interaction between N irrigationus tabaco and VAM fungus, G. lomus mosseae, showed that there was transient enhancement of both enzymatic activities in the inoculated plant roots coinciding in time with the stage of appressoria formation in the root surface. The analysis of free salicylic acid content in roots revealed that the increases in enzymatic activities were coincidental with the accumulation of salicylic acid in inoculated rootst These data indicate that the first reaction of the root cells to the invasion of VAM is a defence response (Billou, Bueno, Ocampo, et al. 2000).

**Effect of ectomycorrhizal fungi on peroxidases and polyphenol oxidases**

In studies conducted at the Department of Botany, University of Wyoming, Laramie, Wyoming, U SA, simple sensitive colorimetric assays for saprotrophic enzyme activity in ectomycorrhizal and saprotrophic Basidiomycetes were derived for in vivo growth situations. Assays were developed for phenol oxidase enzymes including laccase, tyrosinase, peroxidase for cellulase, phosphatase and protease. Enzymatic activity was visualized by placing glass fiber filter paper soaked with agar containing one of several reaction mixtures in contact with ectomycorrhizal root systems and the mycelia of saprotrophic fungi. The types of the reaction mixtures were used depending on the enzyme to be visualized. Assays for phenol oxidase activity were developed by adding specific precursors such as l-dopa, o-tolidine or spring aldehyde to the reaction mixture. These precursors were altered to a coloured product immediately upon exposure to an appropriate enzyme. The coloured patterns from the assay were compared with the original fungal growth to determine locations of enzyme activity. Cellulase and phenoloxidase activity was distributed over the entire mycelium of the saprotrophic fungi tested whereas in ectomycorrhizal fungi, maximum activity occurred near the growing hyphal front and away from ectomycorrhizae. These patterns of enzyme activity have been extremely helpful in formulating hypotheses concerning natural functioning of saprotrophic enzyme systems in ectomycorrhizal fungi and are more useful than in vitro growth studies (Miller 1993).

In studies conducted at the Escola Superior Agrana de Braganca, Apt 172-5300 Braganca Portugal, peroxidase, B-glucosidase and polyphenol oxidase enzymes were discussed to be enzymes responsible for the synthesis of lignin as ectomycorrhization of plant roots must involve cell layers to form the Hartig net. Differences in the activities of these enzymes can indicate how the plant reacts to symbiotic association in terms of lignin synthesis and cell wall construction. The enzymes can allow to distinguish the physiological response of C asatnea sativa and Q uercus robur to a symbiont, Pisolitius tinctorum. The activity of polyphenol oxidase was quantified on an oxygen electrode and the activities of peroxidase and B-glucosidase were determined spectrophotometrically. In preliminary results, the activity of polyphenol oxidase was lower in mycorrhized roots and leaves than in non-mycorrhized ones. The activity of this enzyme after activation with CaCl2 (calcium chloride) and SDS (sodium dodecyl sulphate) was higher on mycorrhized plants (roots and leaves). The ratio between the activities after activation by CaCl2, and SDS was the same for mycorrhized and non-mycorrhized plants. Under the conditions used, the activity of peroxidase was very low for both mycorrhized and non-mycorrhized plants. However, according to the results obtained, it could be suggested that mycorrhized plants presented a higher activity of peroxidase (roots and leaves). After two weeks of incubation, B-glucosidase activity was higher in the roots and leaves of mycorrhized plants than in non-mycorrhized plants (Martins, Keller, Pais, et al. 1993).

Studies conducted at the Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK, on FHOM (fermentation horizon organic matter), collected from a birch forest and placed in transparent observation chambers with mycorrhizal or non-mycorrhizal plants of Betula pendula infected in roots with Paxillus showed that the fungus colonized the FHOM of the mycorrhizal chambers. The activities of protease and polyphenol oxidase increased in organic matter that had been occupied for 28-50 days, and remained elevated in FHOM that had been occupied for 50-98 days. No difference in peroxidase activity was detected between colonized and uncolonized organic matter (Gray and Read 1995).
Studies conducted at the University of Jena, Institute of Microbiology, Philosophenweg, Jena, Germany, the formation of oxidases and peroxidases by newly isolated ectomycorrhizal fungi was examined with agar spot tests and the Bavendamm test for tyrosinase, laccase, polyphenol oxidase, and peroxidase. Extracellular peroxidase was released by virtually all isolates. Tyrosinase (taken as cresolase) was presumably extracellular, although in some ectomycorrhizal genera, the intracellular laccase seemed to dominate. Polyphenol oxidase, as the catecholase/monophenol monooxygenase complex, was found in all isolates although its detection was complicated in the presence of laccase. Lactarius and Russula and the possibly more saprotrophic M orchel showed the most intense enzyme reactions. A comparison with wood and litter decaying fungi indicated that at least some species of several ectomycorrhizal genera possessed extracellular oxidative enzymes that were normally characteristic of white rot fungi. Mycorrhizal fungus grown asymptomatically can release extracellular enzymes capable of oxidizing a wide range of aromatic compounds (Gramss, Gunther, and Frätsche 1998).

Further studies conducted at the above Institute on ectomycorrhizal fungi, Suillus granulatus and Paxillus involutus, grown in both L.C (liquid culture) and in symbiosis with P. sylvestris seedlings under sterile conditions, L.C mycelia of S. granulatus showed intracellular tyrosinase, laccase, and peroxidase activities. In the culture fluid, extracellular laccase and tyrosinase activities were found. P. involutus predominantly produced intracellular laccase. L.C mycelia of S. granulatus suspended in buffer oxidized a range of phenolic acids and mono- and di-phenolic compounds by intracellular mechanism more effectively than did mycelia of P. involutus. In addition, LC mycelia of S. granulatus oxidized p-cresol also by an extracellular mechanism. In symbiosis with Scots pine, S. granulatus and P. involutus increased the level of peroxidase in the fungus/root homogenate and in the nutrient solution of the mycorrhizal plants. Polyphenol oxidase activities were only found with S. granulatus as the mycorrhizal symbiont, and were possibly responsible for the high rate of p-cresol oxidation by this mycorrhizal association. The fungal phenol oxidizing enzymes of ectomycorrhizas can thus substantially contribute to the humification and detoxification processes in soil (Gunther, Perner, and Gramss 1998).

Studies were conducted at the University of Helsinki, Department of Biosciences, Division of Plant Physiology, Helsinki, Finland, nine short-root-specific proteins from Scots pine (P. sylvestris) detected and isolated as individual spots by 2D-PAGE (two dimensional polyacrylamide gel electrophoresis) were identified. The similar peptide mass maps obtained for all nine polypeptide spots together with lectin-blots results suggested that they represented forms of the same modified protein. N-terminal sequence analysis of two of the peptides showed high similarity to peroxidases. RT-PCR (reverse transcription polymerase chain reaction) with oligonucleotide primers corresponding to determined peptide sequences and conserved regions in plant oxidases led to isolation of PSY P1 cDNA which was most abundantly expressed in short roots. PSY P1 was the first peroxidase cDNA to be isolated from the genus Pinus. It encodes a 363 amino acid class-III peroxidase with a calculated molecular mass of 35.7 kDa and theoretical pi of 4.74. The predicted PSY P1 amino acid sequence was grouped with other class-III peroxidases in phylogenetic analyses, but it had a unique amino acid sequence which might be associated with its function in short roots or with its phylogenetic group. The presence of a signal sequence for extracellular transport indicated that PSY P1 belonged to the group of secreted class-III peroxidases. The presence of 10 tyrosine residues and putative auxin-binding regions in PSY P1 suggested that the function of the enzyme was associated with cell-wall formation in short roots. The down regulation of PSY P1 expression in symbiotic short roots hosting the ectomycorrhizal fungus, Suillus bovinus was perhaps related to the change in cell-wall structure necessary for ectomycorrhizal development (Tarkka, N yman, K alkkinen, et al. 2001).

Studies were conducted at the University of Sydney (N epaa), M nanalysis Research Group, School of Science, 10, Kingswood, N. SW, Australia, to verify whether ericoid and ectomycorrhizal fungi produced peroxidase activity as several reports attested to the
apparent ability of some ectomycorrhizal and ericoid mycorrhizal fungi to produce peroxidase enzyme activities during growth in axenic culture. In a critical review of the data, the authors highlighted that peroxidase activities had been apparently observed during growth in media containing 60–70 µM Fe (iron). Ectomycorrhizal and ericoid mycorrhizal fungi are known to produce H$_2$O$_2$ (hydrogen peroxide) via carbohydrate oxidase activity and conditions in common culture media are favourable to the production of hydroxyl radicals, superoxide radicals, and ferryl ions via the Fenton reaction. Free radicals so produced could mediate oxidation of substrates commonly used in presumptive peroxidase assays, leading to false-positive results. It was thus argued that there was currently no evidence to support production of peroxidase activity by ecto- or ericoid mycorrhizal fungi (Cairney and Burke 1998).

**Production of phytoalexins by VAM fungi**

In experiments conducted at the University of Basel, Department of Botany, Hebelstr. 1, Basel, Switzerland, roots of soybean seedlings were brought into close contact with plant roots, well established with VA mycorrhiza (G. mosseae), with root pathogens (Rhizoctonia solani) or with roots of non-infected control plants. Special growth containers were constructed which could be joined or disconnected again to allow to bring together different root systems without disturbing them. In the joined containers, the root systems were separated only by nylon net (60 µm mesh) through which fungal hyphae passed but not the roots. The set up permitted a rapid and even an infection of the seedlings' roots for a period of 30 days. A significantly increased content of phytoalexin (glyceollin) was found in R. solani-infected roots as compared to uninfected control roots. However, there was no difference in the glyceollin contents of the mycorrhizal and control roots for up to 23 days after inoculation. The accumulation of glyceollin in R. solani-infected roots was not influenced by a subsequent inoculation with G. mosseae. Moreover, glyceollin accumulated in mycorrhizal plants to the same extent as in control plants when they were inoculated with R. solani. The two fungi did not mutually influence the course of infection when they were inoculated together (Wys and Wiemken 1988; Wys, Boller, and Wiemken 1991). Studies were conducted at the Plant Biology Division, Samuel Roberts Noble Foundation, Ardmore, OK, USA, on the pterocarpan phytoalexins of the L. eugenioides (F. abacasa), synthesized from L.-phenylalanine via a minimum of 11 enzymatic steps involving the central phenylpropanoid pathway, 3 reactions of flavonoid biosynthesis, and the isoflavonoid branch pathway. The extractable activities of all these enzymes and of enzymes supplying precursors from primary metabolism increased in response to fungal infection or exposure of plant cells to elicitor macromolecules isolated from the cell walls of yeast or pathogenic fungi. The involvement of reductases (oxidoreductases) and cytochrome P450 hydroxylases places a high demand for NADPH (nicotinamide adenine dinucleotide phosphate) on elicited cells. The NADPH is most likely supplied by activation of the pentose phosphate pathway. Genes or cDNAs encoding 7 of the enzymes involved in the synthesis of the phytoalexin medicarpin have been cloned from lucerne and other species. Induction of enzyme activity results from transcriptional activation of the corresponding genes, leading to increased steady state levels of translatable mRNAs. This transcriptional activation is programmed through the interaction of sets of elicitor/infection-modulated transcription factors with their cognate cis elements in the promoters of phytoalexin biosynthetic genes. Gene activation occurs through generation of intracellular signals, which lead to modulation of transcription factor activity, through either increased synthesis of the factor(s), activation via reversible post-translational modification (like phosphorylation/dephosphorylation), translocation of factors from cytoplasm to nucleus or combinations of these. Coordinated induction of the enzymes of phytoalexin synthesis may involve multiple signals and factors for transcriptional activation, as well as feedback and feed-forward fine controls at both transcriptional and post-transcriptional levels. In beneficial mycorrhizal interactions, induction of early pathway genes is uncoupled from that of later phytoalexin-specific genes (Dixon, Harrison, and Paiva 1995).

Studies were conducted at the Department of Mycobiology, School of Biology, M. adurai Kamaraj University, M. adurai, Tamil Nadu, India, on the accumulation of phytoalexin in cowpea during the infection of G. mosseae. Glomus aggregatum or both showed that the percentage of infection and accumulation of phytoalexin were 75%, 62%, and 81% and 220%, 138%, and 280% over the control with respect to G. fasciculatum, G. aggregatum and dual inoculation respectively (Lakshmanan, Gunasekaran, Balse, et al. 1988; Gunasekaran, Balse, Sundaresan, et al. 1988).

**Production of antifungal compounds by ecto-mycorrhizal fungi**

In studies conducted at the Centre de Recherche en Biologie Forestière, Faculté de Foresterie et de Géomatique, Université Laval, Sainte-Foy, Quebec,
were shown to inhibit spore germination and cause metabolites, and a few structurally related compounds, the growth culture of hydroxyethanoic acid, pisolithin B) were isolated from hydroxymandelic acid, ((R)-(−)-2-(4′-hydroxyphenyl)-2-oxoethanoic acid, pisolithin A) and (R)-(−)-o-p-hydroxybenzoylformic acid (2-(4′-hydroxyphenyl)-2-oxoethanoic acid, isolated from P. tinctorius was shown to have the absolute (R) configuration. The stereochrometry was established via comparison of its optical rotation with that of its synthetic (R) and (S) enantiomers. The synthetic samples were prepared by the stereospecific reduction of the prochiral alpha-keto acid, p-hydroxybenzoylformic acid (2-(4′-hydroxyphenyl)-2-oxoethanoic acid), with (R) or (S)-2, 2′-dihydroxy-1, 1′-binaphthyl lithium aluminium hydride (BINAL-H). The absolute configuration and enantiomeric purity of both products were determined using the 1H NMR of their isobutyl esters in the presence of the chiral solvating agent (R)-(−)-2,2,2-trifluoro-1-(9-anthryl) ethanol (Tsantrizos and Ogilvie 1991).

Studies were conducted at the Dipartimento di Scienze Ambientali, University of L’Aquila, Italy, on the effects of truffle metabolites on the rhizospheric environment. Several species of Tuber produce a ‘burned’ area around their symbiotic plants as a result of phytotoxic action. The volatile substances from Tuber sporophores, truffles, had been previously identified by gas chromatography coupled to gas mass spectrometry and then tested for their toxic effects on higher plants as well as soil micro-organisms. All tested organisms appeared to suffer a great inhibition when exposed to some of these substances evaporated into desiccators. Three aldehydes (2-methyl propanal, 2-methyl butanal, and 3-methyl butanal) produced these effects on the two soil fungi tested (Penicillium vinaceum and A. gypseum). In addition to some of these, two alcohols (2-methyl butanol and 3-methyl butanol) were also active on plants (Triticum vulgare and Lens culinaris). 2-M ethyl butanol generally affected plants to a high degree, but the strongest effects to micro fungi were caused by 3-methyl butanal. However, a strain of Pseudomonas isolated from truffle surface tolerated much higher concentrations of the three aldehydes. The substances affecting the soil micro-aerobic environment, where seeds germinate and roots live, are normal compounds of fungal metabolism produced via pyruvate pathway. Their toxic action could be a consequence of alterations caused both to the cellular membrane and to nucleic acids. The new term ‘hydnosphere’ was proposed to define this peculiar case of soil relationships between truffle and neighbouring coenosis (Pacioni 1991).

Studies conducted at the Graduate Program in Plant Physiology, The Pennsylvania State University,
University Park, USA, on production of antifungal compounds by roots of mycotrophic (hosts of VAM fungi) and non-mycotrophic (non-hosts) families showed that among non-mycotrophic species, five species from the Brassicaceae (Brassica kaber, Brassica napus, Brassica campestris, Thlaspi arvense, Raphanus raphanistrum) had detectable antifungal compounds in extracts from roots. Two species from the Chenopodiaceae (Spinacea oleracea, Beta vulgaris) and one species from A maranthaceae (A maranthus retroflexus) did not have any antifungal compounds. One mycotrophic species (Daucus carota) also had a detectable antifungal compound in root extracts, while three other mycotrophic species (Lactuca sativa, Abutilon theophrasti, and Sorghum bicolor) did not. Chloroform extracts of B. kaber roots appeared to have the greatest quantity of extractable antifungal compounds as determined by the TLC bioassay. In a separate experiment, chloroform extracts from B. kaber roots greatly inhibited the germination of spores of Glomus etunicatum, while extracts from a number of other mycotrophic and non-mycotrophic plant roots were only marginally inhibitory. Three antifungal compounds in chloroform extracts from B. kaber roots were derived from glucosinolates. The predominant antifungal compound was identified as 4-hydroxybenzyl isothiocyanate, the isothiocyanate of p-hydroxybenzoylformic acid and (R)-(-)-p-hydroxymandelic acid, two antifungal compounds produced by the action of myrosinase on glucosinolabin. The data provided evidence of a possible role for myrosinase on glucosinolates in determining the non-mycotrophic status in B. kaber (Schreiner and Koid 1993).

References

Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the arbuscular mycorrhizal Glomus mosseae
Mycological Research 104: 722–725

Cairney J W G and Burke R M. 1998
Do ecto- and ericoid mycorrhizal fungi produce peroxidase activity?
Mycorrhiza 8(2): 61–65

The isoflavonoid phytoalexin pathway: from enzymes to genes to transcription factors
Physiologia Plantarum 93(2): 385–392

Ezz T and Nawar A. 1994
Salinity and mycorrhizal association in relation to carbohydrate status, leaf chlorophyll, and activity of peroxidase and polyphenol oxidase enzymes in sour orange seedlings

Spot tests for oxidative enzymes in ectomycorrhizal, wood, and litter decaying fungi
Mycological Research 102: 67–72

Qualitative and quantitative characterisation of phenolic derivatives likely involved in the pathogen resistance of endomycorrhizal roots
Wyoming: University of Wyoming, p. 123

[Eight North American Conference on Mycorrhiza Innovation and Hierarchical Integration, Jackson, Wyoming, USA, 5–8 September 1990, organized by University of Wyoming and San Diego States University]

Grandmaison J, Olah G M, Calsten M R, Furlan V. 1993
Characterization and localization of plant phenolics likely involved in the pathogen resistance expressed by endomycorrhizal roots
Mycorrhiza 3: 155–164

Gray B D and Read D J. 1995
The structure and function of the vegetative mycelium of ectomycorrhizal plants
New Phytologist 130(3): 411–417

Gunasekaran P, U balthoose Raja N, Sundaresan P, Lakshmanan M. 1988
Synthesis of phytoalexin during mycorrhizal association in cowpea
Florida: University of Florida
[Seventh North American Conference on Mycorrhizaes, Gainesville, Florida, 3–8 May 1987, organized by University of Florida]

Gunther H, Perner B, and Gramss G. 1998
Activities of phenol oxidizing enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (Pinus sylvestris L.)
Journal of Basic Microbiology 38(3): 197–206

p-Hydroxybenzoylformic acid and (R)-(−)-p-hydroxymandelic acid, two antifungal compounds isolated from the liquid culture of the ectomycorrhizal fungus Pisolithus arhizus
Canadian Journal of Microbiology 37(4): 258–264

Lakshmanan M, Gunasekaran P, U balthoose R N, Sundaresan P. 1988
Production of phytoalexin in cowpea infected with VA mycorrhizal fungi, p. 627
In M mycorrhiza Round T able: Proceedings of a workshop, pp. 530–534
New Delhi: India
[JNU-IDRC, Canada Sponsored National Workshop on Mycorrhizae, New Delhi, India, 13–15 March 1987]
Study of the enzymatic activity of PPO
(Polyphenoloxidase), PO (Peroxidase), and B-Glu
(B-Glucosidase) on mycorrhized and non-
mycorrhized plants
In Proceedings of the Ninth North American Conference on
M ycorrhizae
Ontario: University of Guelph. p. 83
[Ninth North American Conference on M ycorrhizae,
Ontario, USA, 8–12 August 1993, organized by University
of Guelph]

M athur N and Vyas A. 1995
Changes in isozyme patterns of peroxidase and
polyphenol oxidase by VAM fungi in roots of
Ziziphus species

M iller S L. 1993
Visualization and localization of saprotrophic
enzyme activity in vivo in ectomycorrhizal and
saprotrophic basidiomycetes
In Proceedings of the Ninth North American Conference on
M ycorrhizae
Ontario: University of Guelph. p. 10
[Ninth North American Conference on M ycorrhizae,
Ontario, USA, 8–12 August 1993, organized by University
of Guelph]

M unzenberger B Z. 1993
Reduction of plant-produced phenolics in ecto- and
ectendomycorrhizas - a requirement for
mycorrhization?
In Proceedings of the Ninth North American Conference on
M ycorrhizae
Ontario: University of Guelph. p. 11
[Ninth North American Conference on M ycorrhizae,
Ontario, USA, 8–12 August 1993, organized by University
of Guelph]

M unzenberger B, Hilemann J, Strack D, Kottake I,
Oberwinkler F. 1990
Phenolics of mycorrhizas and non-mycorrhizal
roots of Norway spruce
Planta 182(1): 142–148

M unzenberger B, Kottke I, and Oberwinkler F. 1995
Reduction of phenolics in mycorrhizas of Larix
decidua Mill
Tree Physiology 15(3): 191–196

N agahashi G, Abney G D, and Doner L W. 1996
A comparative study of phenolic acids associated
with cell walls and cytoplasmic extracts of host and
non-host roots for AM fungi
New Phytologist 133(2): 281–288

Pacioni G. 1991
Effects of Tuber metabolites on the rhizospheric
environment
Mycological Research 95(12): 1355–1358

Schreiner P R and Koide R T. 1993
Antifungal compounds from the root of
mycotrophic and non-mycotrophic plant species
New Phytologist 123: 99–105

Selvaraj T and Subramanian G. 1990
Phenols and lipids in mycorrhizal and non-
mycorrhizal roots of Sessamum indicum
Current Science 59(9): 471–473

Spami P and Bonfante-Fasolo P. 1988
Cell-wall-bound peroxidase activity in roots of
mycorrhizal Allium porrum

Sword M A and Garrett H E. 1994
Boric acid-phenolic relationships within the Pinus
echinata–Pisolithus tinctorius ectomycorrhizal
association
Tree Physiology 14(10): 1121–1130

T am C F Paul and Griffiths D A. 1993
Mycorrhizal associations in Hong Kong Fagaceae
M ycorrhiza 3:165–172

Tarkka M T, Nyman T A, Kalkkinen N, Raudaskoski
M., 2001
Scots pine expresses short-root-specific peroxidases
during development

Antifungal antibiotics from Pisolithus tinctorius
Phytochemistry 30(4): 1113–1118

Tsantrizos Y S and Ogilvie K K. 1991
Determination of the absolute stereochemistry of the
fungal metabolite (R)-(−)-2-(4'-hydroxyphenyl)-2-hydroxyethanoic
acid (pisolithin B)
Canadian Journal of Chemistry 69(5): 772–778

W yss P, Boller T., and Wiemken A. 1991
Phytoalexin response is elicited by a pathogen
(Rhizoctonia solani) but not by a mycorrhizal
fungus (Glomus mosseae) is soybean roots
Cellular and M olecular Life Sciences 47(4):395-399

W yss P and Wiemken A. 1988
Comparison of reactions in plant roots induced by
infection by pathogenic and VA mycorrhizal fungi
[Second European Symposium on M ycorrhizae.
14–20 August, 1988. Prague, Czechoslovakia]
Research finding papers

Phosphatase activity in the rhizosphere of medicinal plants inoculated with arbuscular mycorrhizal fungi

Anuj Kumar Singh and Jamaluddin*
Tropical Forest Research Institute, Jabalpur – 482 021, Madhya Pradesh, India

* e-mail: jamaluddin_125@hotmail.com; anuj_30680@yahoo.com

Introduction
Phosphorus is one of the least available plant nutrient found in the soil. A significant amount of phosphate is bound in organic forms in the rhizosphere. Phosphatases produced by plants and microbes are responsible for converting organic phosphorus into available phosphate, which is absorbed by plants.

Phosphate availability is one of the major growth limiting factors for plants in many natural ecosystems. In response to persistent phosphate deficiency, plants develop many adaptive mechanisms to enhance the availability and uptake of phosphate. One such adaptive mechanism is the production and secretion of phosphatases to release phosphate from organic forms (Goldstein, Danon, Baertlein, et al. 1988).

It is also known that phosphatases catalyse the hydrolysis of several organic phosphate monoesters, liberating available phosphate in the soil. The activity of this enzyme is an important physiological characteristic related to plant efficiency in relation to phosphorus mobilization (Tadano, Ozawa, Sakai, et al. 1993).

The present study aims to assess the phosphatase (Pase) enzyme activity in the rhizosphere soil of two important medicinal plants like Rauvolfia serpentina Benth. and Coleus forskohlii Briq. inoculated with AM (arbuscular mycorrhizal) fungi.

Materials and methods
To assay the phosphatase enzyme activity, the method of Tabatabai and Bremner (1969) was followed. This method was followed throughout the investigation starting from collection of soil samples. The rhizosphere soil of R. serpentina and C. forskohlii were collected from both inoculated and uninoculated plants that were grown in sterilized soil.

The soil samples so collected were cleaned and 1 g of dried soil (less than 2 mm) of each sample was placed in a 50 ml wide mouthed test tube and 0.2 ml of toluene, as well as 4 ml of M U B (modified universal buffer), were added to it. Subsequently 1 ml of p-nitrophenyl phosphate, prepared in the same buffer (pH 6.5 for assay of acid phosphatase and pH 11.0 for assay of alkaline phosphatase), was added. The test tubes were stoppered and placed in an incubator at 37 °C for 1 hour. After incubation, the stoppers were removed and 1 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH were added; and the test tubes were swirled for few seconds. The soil suspension was filtered through a Whatman No. 2. The colour intensity of the filtrate was measured spectrophotometrically at 420 nm. Calculation of the p-nitrophenol content of the filtrate was made using the reference of a calibration graph, plotted from results obtained with standards containing 0, 10, 20, 30, 40, and 50 µg of p-nitrophenol, and the results were expressed in terms of µg p-nitrophenol released per g soil.

Recovery of viable VAM (vesicular arbuscular mycorrhizal) spores was made using the method of Gerdemann and Nicolson (1963). Staining of fine roots was processed by following the method of Phillips and Hayman (1970).

Results and discussion
All plants require phosphorus, in large amounts, for their growth and development; as it is a constituent element of many specific compounds that make up the plants’ structure and play an important part in metabolic processes.

Phosphorus is abundant in soils, both in organic and inorganic forms. It is often a major or even the prime-limiting factor for plants growth. Enhanced phosphatase activity may help plants acquire the required amount of phosphate from the rhizosphere (Raghothama 1999). A combination of phosphatase activity and enhanced phosphate uptake may help plants acquire the required amount of phosphorus from the rhizosphere.

* e-mail: jamaluddin_125@hotmail.com; anuj_30680@yahoo.com
The data generated by this study (Table 1), shows that the acid phosphatase (APase) activity was highest at 188.0 µg in the rhizosphere soil of VAM inoculated R. serpentina, while it was only 74.0 µg in case of uninoculated plants. It was also observed that alkaline phosphatase (ALPase) activity was higher (78.0 µg) in the rhizosphere soil of inoculated R. serpentina plants as compared to uninoculated plants (67.0 µg).

In case of C. forskohlii, acid phosphatase activity was higher (46.0 µg) in the rhizosphere soil of VAM inoculated plants as compared to uninoculated plants (29.5 µg). Alkaline phosphatase activity was also higher (46.5 µg) in rhizosphere soil of inoculated plants than rhizosphere soil of uninoculated (26.5 µg) plants.

It is seen that APase activity was found higher than ALPase activity in inoculated plants of both the species. In case of C. forskohlii, there was no considerable difference observed between APase and ALPase activity in the rhizosphere soil of inoculated plants; while in uninoculated plants, the APase activity (29.5 µg) observed was higher than the ALPase activity (26.5 µg). Krishna and Bagyaraj (1985) also noted increased phosphatase activity in rhizosphere of mycorrhizal plants. Results in the similar fashion were also observed with papaya (Mohandas 1990).

Percentage of root infection and VAM spore population density were found to be higher in R. serpentina roots and rhizosphere soil as compared to C. forskohlii.

From the above study, it was concluded that the phosphatase enzyme activity is increased in rhizosphere soil collected from plants inoculated with AM fungi.

### References

Gerdemann J W and Nicolson T H. 1963

Spores of mycorrhizal endogone species isolated by wet sieving and decanting

Transactions of the British Mycological Society 46: 235–244

Goldstein A H, Danon A, Baertlein D A, M c D a n i e l R G. 1988

Phosphate inducible metabolism in Lycopersicon esculentum: characterization of acid phosphatase by tomato and suspension cultured cells I

Plant Physiology 87: 716–720

Krishna K R and Bagyaraj D J. 1985

Phosphatases in the rhizosphere of mycorrhizal and non-mycorrhizal groundnut


Mohandas S. 1990

Enhanced phosphatase activity in mycorrhizal papaya (Carica papaya cv. Coorg Honey Dew) roots

In Current Trends in Mycorrhizal Research, pp. 55-56, edited by B L Jalali and H Chand

India. 213 pp.


Improved procedures for clearing root and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection

Transactions of the British Mycological Society 55:158–161

Raghothama K G. 1999

Phosphate acquisition

Annual Review of Plant Physiology and Molecular Biology 50: 665–693

Tabatabai M A and Bremner J M. 1969

Use of p-nitrophenyl phosphate for assay of soil phosphatase activity

Soil Biology and Biochemistry 1: 301–307

Tadano T, Ozawa K, Sakai H, Osaki M, M at su i H. 1993

Secretion of acid phosphatase by the roots of crop plants under phosphorus deficient conditions and some properties of the enzyme secreted by lupin roots

Plant and Soil 155-156(1): 95–98

---

**Table 1** Phosphatase activity and arbuscular mycorrhizal infection in two important medicinal plants

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Phosphatase activity</th>
<th>Root infection (%)</th>
<th>VAM spore population density/100 g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid phosphatase</td>
<td>Alkaline phosphatase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inoculated (µg)</td>
<td>Uninoculated (µg)</td>
<td>Inoculated (µg)</td>
</tr>
<tr>
<td>R. serpentina</td>
<td>188.0</td>
<td>74.0</td>
<td>78.0</td>
</tr>
<tr>
<td>C. forskohlii</td>
<td>46.0</td>
<td>29.5</td>
<td>46.5</td>
</tr>
</tbody>
</table>

VAM – vesicular arbuscular mycorrhizal; C. forskohlii – Coleus forskohlii; R. serpentina – Rauvolfia serpentina
Response of Rangpur lime to different endomycorrhizal species for germination, growth parameters, and graft success

P Barman, G S K Swamy, P B Patil, C P Patil, and N T Hammaiah
Department of Pomology, KRC College of Horticulture, Arabhavi – 591 310, Karnataka, India

Introduction

AM (arbuscular mycorrhizal) fungi are known for improving plant growth, mainly through their influence on root geometry with production of roots aided by an increase in an uptake of major nutrients (Adivappar, Patil, Patil, et al. 2004), leading to increased photosynthetic activity. Keeping this in view, the present study was taken up with an objective of knowing the response of Rangpur lime to different AM fungi for germination, growth parameters, and grafting success.

Material and methods

The present investigation was carried out at Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, during 2005/06 to study the germination and growth parameters of Rangpur lime rootstock, as influenced by AM fungi. Rangpur lime seeds were collected from fully matured fruits harvested from healthy mother trees during the month of June. The rootstocks were raised in polybags of 10 × 8 cm (300 gauge) filled with soil: sand: FYM (farm yard manure) in 1: 1: 1 (v/v) proportion. The inoculum used was multiplied raising maize (Zea mays) and ragi (Eleusine coracana) as hosts in pots containing sterilised potting mixture, which consisted of a mixture of sand, soil, and FYM in 1: 1: 1 (v/v) proportion. The inoculum consisted of root segments of maize and ragi comprising of hyphae, vesicles, arbuscules, and chlamydospores of the AM fungi. Inoculation of AM fungi to Rangpur lime was done at five grams per polybag consisting of 80 to 88 infective propagules. The design of the experiment adopted was completely randomized with five treatments (Control, Acaulospora laevis, Glomus mosseae, Glomus bagyaraji and Glomus leptotichum) and four replications. Observation on germination percentage was recorded daily, while the growth parameters were recorded at 180 and 270 DAS (days after sowing). Extrametrical chlamydospores produced by AM fungi were determined by wet sieving and decanting method (Gerdemann and Nicolson 1963) and percent root colonization was determined by using the method as detailed by Phillips and Hayman (1970). Nitrogen content was estimated by Kjeldahl method (Ranganna 1991). Nitrogen percent, estimated in a particular sample, was multiplied by 6.25 to determine protein percent in the corresponding sample.

Results

The results revealed that G. leptotichum inoculated Rangpur lime seeds recorded maximum germination (98.69%) as compared to the control (78.67%) (Table 1). At 180 DAS, A. laevis inoculated rootstock recorded highest height (16.57 cm) as compared to the control (9.03 cm). At 270 DAS, the highest rootstock height was recorded in A. laevis, G. mosseae, G. leptotichum, and G. bagyaraji inoculated rootstocks (21.27 cm, 19.92 cm, 19.76 cm, and 18.63 cm, respectively) as compared to the control (12.17 cm) (Table 1). At 180 DAS, uninoculated rootstocks showed minimum number of leaves, while there was no significant difference with the treatments. At 270 DAS, maximum number of leaves was recorded in G. mosseae, A. laevis, and G. leptotichum inoculated rootstocks (47.73, 45.27, and 41.62, respectively) as compared to the control (21.27) (Table 1). At 180 DAS, the maximum stem diameter was recorded in rootstock inoculated with A. laevis (3.95 mm) as compared to the control and G. bagyaraji inoculated rootstocks (2.28 mm and 2.47 mm, respectively). At 270 DAS, the maximum stem diameter was recorded in rootstock inoculated with G. mosseae and A. laevis (4.84 mm and 4.64 mm, respectively) as compared to the control and G. bagyaraji inoculated rootstocks (2.28 mm and 2.47 mm, respectively). At 270 DAS, the maximum stem diameter was recorded in rootstock inoculated with G. mosseae and A. laevis (3.95 mm) as compared to the control and G. bagyaraji inoculated rootstocks (2.28 mm and 2.47 mm, respectively). At 270 DAS, the maximum stem diameter was recorded in rootstock inoculated with G. mosseae and A. laevis (4.84 mm and 4.64 mm, respectively) as compared to the control and G. bagyaraji inoculated rootstocks (3.81 mm and 3.94 mm, respectively) (Table 1). At 180 DAS, the highest number of lateral roots was recorded in G. leptotichum (39.80 cm), followed by G. mosseae (34.20 cm) as compared to the control (24.60 cm) (Table 1). At 180 DAS, the highest number of lateral roots was recorded in G. leptotichum.
inoculated rootstocks (76.00) followed by G. mosseae (68.00), while the lowest was recorded in the rootstocks inoculated with G. bagyaraji (37.33) and the control (38.33). At 270 DAS, the highest number of lateral roots was recorded in G. leptotichum inoculated rootstocks (120.00) followed by G. mosseae (99.00) and A. laevis (87.00) as compared to the control (68.00) and G. bagyaraji inoculated rootstocks (72.00) (Table 1). G. mosseae inoculated rootstocks recorded significantly the highest nitrogen content in the shoot (3.01% and 2.24%) and in the root (2.59% and 1.12%) at 180 and 270 DAS respectively, compared to other treatments. G. mosseae inoculated rootstocks also recorded highest protein content at 180 and 270 DAS both in shoot (19.29% 14.00%) and in root (16.28% and 7.00%) respectively, as compared to other treatments (Table 2). Significantly maximum number of chlamydospores and percent root colonization was recorded in G. mosseae (966.25/500 g of soil and 95.50%, respectively) (Table 3). The highest graft success was recorded in G. leptotichum inoculated rootstock (95.35%) followed by G. mosseae (90.00%). The highest graft survival was recorded in G. leptotichum and G. mosseae inoculated rootstocks (97.56% and 97.22%, respectively) (Table 3). Percent root colonization, number of chlamydospores/50 g of soil, graft success, and graft survival were found to be positively correlated with each other (Table 4). 

**Discussion**

The improvement in germination of Rangpur lime seeds inoculated with AM fungi could be due to the fact that soon after sowing of the fresh seeds, they start imbibing water as well as leaching several metabolites including amino acids, organic acids, inorganic ions, sugars, phenolics, and protein

---

**Table 1** Effect of arbuscular mycorrhizal fungi on germination and growth parameters of Rangpur lime rootstock

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination (%)</th>
<th>Number of leaves</th>
<th>Rootstock diameter (mm)</th>
<th>Primary root length (cm)</th>
<th>Number of lateral roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180 DAS</td>
<td>270 DAS</td>
<td>180 DAS</td>
<td>270 DAS</td>
<td>180 DAS</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; - Control</td>
<td>78.67</td>
<td>9.03</td>
<td>12.17</td>
<td>17.00</td>
<td>21.27</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; - Acaulospora laevis</td>
<td>87.20</td>
<td>16.57</td>
<td>21.27</td>
<td>29.80</td>
<td>45.27</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; - Glomus mosseae</td>
<td>90.00</td>
<td>16.07</td>
<td>19.92</td>
<td>30.53</td>
<td>47.73</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; - Glomus bagyaraji</td>
<td>86.00</td>
<td>15.29</td>
<td>18.63</td>
<td>25.85</td>
<td>30.73</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; - Glomus leptotichum</td>
<td>98.69</td>
<td>15.56</td>
<td>19.76</td>
<td>28.00</td>
<td>41.62</td>
</tr>
<tr>
<td>S.Em±</td>
<td>1.57</td>
<td>0.95</td>
<td>0.93</td>
<td>1.86</td>
<td>2.58</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>4.72</td>
<td>2.86</td>
<td>2.80</td>
<td>5.62</td>
<td>7.79</td>
</tr>
</tbody>
</table>

**Table 2** Effect of arbuscular mycorrhizal fungi on shoot and root nitrogen and protein percent (on dry weight basis) of Rangpur lime rootstock at various stages of growth

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Nitrogen (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>180 DAS</td>
<td>270 DAS</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; - Control</td>
<td>0.84</td>
<td>0.49</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; - Acaulospora laevis</td>
<td>1.89</td>
<td>1.75</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; - Glomus mosseae</td>
<td>3.01</td>
<td>2.59</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; - Glomus bagyaraji</td>
<td>2.31</td>
<td>2.03</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; - Glomus leptotichum</td>
<td>1.61</td>
<td>0.91</td>
</tr>
<tr>
<td>S.Em±</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>0.17</td>
<td>0.06</td>
</tr>
</tbody>
</table>
These solute leachates are important because they help AM fungal spores to germinate, as there is molecular and chemical dialogues between AM fungi and hosts. In turn, AM fungi help in better seed germination by mutualistic symbiosis with seed and competing with pathogens for space and nutrients. Increased germination percentage due to AM fungal species is reported in Rangpur lime (Venkat 2004) and mango (Santosh, 2004; Bassanagouda 2005).

G. leptotichum was found to be the most efficient AM fungus in increasing root parameters recorded at 180 and 270 DAS. In all the cases, uninoculated stocks produced minimum root parameters. Modifications in the root geometry and morphology might be due to morphogenic effects mediated by IAA (indol acetic acid) and gibberellins (Allen, Moore, and Christensen 1980). AM fungal rootstocks also showed greater height, stem diameter, and more number of leaves. The improvement in rootstock growth and nitrogen and protein content could be attributed to AM fungal association in terms of percent root colonization and spore count. The result of softwood grafting is in agreement with Venkat (2004) who observed that G. leptotichum and G. mosseae inoculated stocks gave the highest graft success percentage. The higher graft survival of G. mosseae inoculated rootstocks might be due to rapid improvement in growth of the rootstock due to higher root colonization and more number of chlamydospores (Gerdemann 1968).

### Table 3  Effect of arbuscular mycorrhizal fungi on percent root colonization and spore count of Rangpur lime rootstocks

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root colonization (%)</th>
<th>Spore count (number of chlamydospores/50 g soil)</th>
<th>Graft success (%)</th>
<th>Graft survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt; - Control</td>
<td>37.50</td>
<td>92.25</td>
<td>61.09</td>
<td>68.18</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt; - Acaulospora laevis</td>
<td>92.50</td>
<td>942.25</td>
<td>85.00</td>
<td>94.12</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; - Glomus mosseae</td>
<td>95.50</td>
<td>966.25</td>
<td>90.00</td>
<td>97.22</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; - Glomus bagyaraji</td>
<td>87.50</td>
<td>853.25</td>
<td>81.25</td>
<td>84.62</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; - Glomus leptotichum</td>
<td>90.50</td>
<td>937.25</td>
<td>95.35</td>
<td>97.56</td>
</tr>
<tr>
<td>S. Em±</td>
<td>0.65</td>
<td>2.63</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>C.D. at 5%</td>
<td>1.94</td>
<td>7.91</td>
<td>2.09</td>
<td>2.09</td>
</tr>
</tbody>
</table>

### Table 4  Correlation studies of microbiological parameters versus graft success and graft survival of Rangpur lime rootstocks treated with arbuscular mycorrhizal fungi

<table>
<thead>
<tr>
<th>Root colonization (%)</th>
<th>Spore count (number of chlamydospores/50 g soil)</th>
<th>Graft success (%)</th>
<th>Graft survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.000</td>
<td>0.999*</td>
<td>0.927*</td>
<td>0.939*</td>
</tr>
<tr>
<td>1.000</td>
<td>0.939*</td>
<td>0.947*</td>
<td>0.976*</td>
</tr>
<tr>
<td>1.000</td>
<td>0.976*</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

*Significant at P = 0.01 level

### References

**Effect of AM fungi on growth and nutrient content of container grown papaya plants**

Allen M F, Moore T S, and Christensen M. 1980
**Phytohormone changes in** Bouteloua gracilis **infected by vesicular arbuscular mycorrhizal cytokinin increase in the host plant**
Canadian Journal of Botany 58: 371–374

Bassanagowda. 2005
**Synergetic effect of AM fungi in combination with bioformulations on germination, graft-take, growth and yield of mango**

Gerdemann J W. 1968
**Vesicular arbuscular mycorrhizae and plant growth**
Annual Review Phytopathology 6: 397–418

Gerdemann J W and Nicolson J H. 1963
**Spores of mycorrhiza Endogone species extracted from soil by wet sieving and decanting**
Transactions of the British Mycological Society 46: 235–244

**Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection**
Transactions of the British Mycological Society 55: 158–161
Vesicular arbuscular mycorrhizal association in Bryophytes isolated from Eastern and Western Himalayas

Deepak Vyas, Archana Dubey, Anuradha Soni, Mahendra K. Mishra, Pradeep K. Singh, and R. K. Gupta*

Laboratory of Microbial Technology and Plant Pathology, Department of Botany, Dr H S Gour University, Sagar (Madhya Pradesh)

Introduction

Bryophytes established a significant and important group in the plant kingdom. Mosses, the largest group of bryophytes, are generally non-mycorrhizal, but often contain endohyphae of VAM (vesicular arbuscular mycorrhizal) fungi (Rabatin 1980; Turnau, Ronikier, and Unrug 1999). Liverworts and hornworts have VAM-like association with glomalean fungi that form arbuscules in their thalli. Fine endophytes (glomalean fungi with very narrow hyphae) forming VAM with arbuscules are common in bryophytes, but other VAM fungi such as Glomus sp. are also present (Johnson 1977; Turnau, Ronikier, and Unrug 1999; Schubler 2000). The first land plants, structurally very weak (Kenrick and Crane 1977), needed to be highly permeable to acquire water nutrients.

Bryophytes occupy an important ecological niche (moist habitat) and harbour many nutrients. Prompted with this quality of bryophytes, the present study was undertaken.

Materials and methods

The senior author had collected the samples of bryophytes along with rhizospheric soil in January 2004 and January 2005 from two sites; site I – Eastern Himalayas, Darjeeling, West Bengal and site II – Western Himalayas, Musoorie, and Uttranchal.

Site I has an average annual rain fall of 100–300 cm, relative humidity 83%, and annual average temperature 11–14 °C. The height of Darjeeling from MSL (mean sea level) is 7120 feet. Site II has an average annual rain fall 180 cm, relative humidity 80%, average temperature 10–20 °C and the height from the MSL 7200 feet.

The thalli were completely uprooted. Extraction of VAM fungal spores from rhizospheric soil was done in our laboratory at Department of Botany, Dr H S Gour University, Sagar, following the method of wet sieving and decanting (Gerdemann and Nicolson 1963).

Spore population was calculated per 100 g of dry soil. The ventral rhizoidal part of the thallus was stained in case of genera of Hepaticopsida. In others, rhizoids were stained and mounted using the method of Phillips and Hayman (1970). Here we used 10% NaOH in place of KOH solution. Soil analysis was done as per M. Ishra (1968). pH and conductivity were determined as per method of Cromwell (1955). Nitrogen and potassium were analyzed as per method of Jackson (1973). Phosphorus and base deficiency were measured by the method of M. Ishra (1968). Per cent organic carbon was measured by the method of Piper (1944).
Results

Table 1 shows the data on percent colonization, spore population, presence of fungal structures, and distribution of VAM species. It is clearly evident from the results that hyphal colonization was present in all the eight collected bryophytes. Arbuscular and vesicular structures are seen in Pellia and Marchantia.

Data depicted in Table 1 are of both sites—site I and II. It is clearly evident that bryophytic flora collected and identified from site I belongs to all the three classes of bryophyta, that is Hepaticopsida, Anthropsida, and Bryopsida. The results obtained from the experiments suggest that Pellia sp, which belongs to the order Jungermaniaceae of Hepaticopsida class, not only shows a greater number of spores (1289/100 g of dry soil), but also has maximum colonization (75%) and harboured maximum species (eight) of VAM fungi. Pellia is the only genus of this site where we observed arbuscules and vesicles. The minimum number of spores (960/100 g of dry soil), lesser colonization (45%), and only three VAM species were recorded in Funaria. Whereas in Riccia sp1 and Notothyllus, the number of spores recorded was 1120 and 1092/100 g of dry soil, and percent root colonization was 65% and 54% respectively, though the number of VAM fungi was the same, yet species were totally different. All these genera do not have any arbuscular or vesicular structures.

The bryophytic flora collected from site II belongs only to class H epaticopsida. A larger number of VAM fungal spores was recorded in Riccia sp; that is 1244/100 g of dry soil, followed by 1169 in Plagiochasma sp. and 1112 in M archantia sp. and least 810 were recorded with A strella sp. M aximum 60% colonization was also observed with Riccia sp and minimum 40% colonization was recorded with A strella sp. M archantia shows 55% and Plagiochasma shows 50% colonization. Similarly maximum seven VAM species was found with Riccia sp followed by six sp. in M archantia sp. Four and two VAM sp. were recorded in Plagiochasma and A strella, respectively. As mentioned earlier, that arbuscules and vesicles were observed in M archantia sp.

Table 2 shows the data of physico-chemical properties of soil samples collected from the test sites I and II. It is evident from the results that soil of site I was slightly alkaline whereas site II soil was found acidic. Greater conductivity, brown colour, loamy texture, and volatile odour was recorded in the

Table 1 VAM fungal association with Bryophytes in two different Himalayas region.

<table>
<thead>
<tr>
<th>Name of Bryophytes</th>
<th>Colonization (%)</th>
<th>Spore population/100 g soil</th>
<th>Presence of fungal strains</th>
<th>Number of VAM species</th>
<th>Occurrence of VAM fungal species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riccia sp.</td>
<td>65</td>
<td>1120</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Pellia sp.</td>
<td>75</td>
<td>1289</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Notothyllus sp.</td>
<td>54</td>
<td>1092</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Funaria sp.</td>
<td>45</td>
<td>960</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Riccia sp.</td>
<td>60</td>
<td>1244</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Marchantia sp.</td>
<td>55</td>
<td>1162</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Plagiochasma sp.</td>
<td>50</td>
<td>1129</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Asterella sp.</td>
<td>40</td>
<td>810</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

VAM – vesicular arbuscular mycorrhizal; LMGC – Glomus magnicaule (Hall); GABD – Gigaspora albida (Schenck and Smith); ASCB – Acaulospora scrobiculata (Trappe); ANCS – A. nicolsonii (Walker, Read, and Sanders); ASPN – A. spinisa (Walker); LFSC – Glomus fasciculatum (Thaxter Sensu Gerd.); LMSS – Glomus mosseae (Nicol. and Gerd.); LOCT – Glomus occultum (Walker); LPST – Glomus pustulatum (Koske, Fries, Walker, and Dalpe); LDMR – Glomus dimorphicum (Boyetchko and Tewari); LABS – Glomus ambisporum (Smith and Schneck); LMSS – Glomus magniculae (Hall); Species code as per Perez and Schenck (1990).

Table 2 Physico-chemical analysis of study sites I and II

<table>
<thead>
<tr>
<th>Site</th>
<th>ph</th>
<th>Conductance</th>
<th>N (kg/h)</th>
<th>P (kg/h)</th>
<th>K (kg/h)</th>
<th>C (%)</th>
<th>Base deficiency</th>
<th>Colour</th>
<th>Texture</th>
<th>Odour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site I Darjeeling</td>
<td>7.8</td>
<td>0.30</td>
<td>296</td>
<td>10.4</td>
<td>325</td>
<td>4.4</td>
<td>+</td>
<td>Brown</td>
<td>Loamy</td>
<td>Volatile</td>
</tr>
<tr>
<td>Site II Mussoorie</td>
<td>6.5</td>
<td>0.24</td>
<td>360</td>
<td>8.0</td>
<td>198</td>
<td>2.4</td>
<td>++</td>
<td>Gray</td>
<td>Sandy</td>
<td>Ammonium</td>
</tr>
</tbody>
</table>

+ - highly base deficient; ++ - less base deficient; K – Potassium; P – Phosphorus; N – Nitrogen; C – organic Carbon
soil of site I. Whereas soil of site II showed lesser conductivity, grey colour, sandy texture, and ammonium odour respectively. Nitrogen was recorded less in soil sample of site I, but phosphorus, potassium, and percent organic carbon was recorded higher in same sample. Whereas contrasting results are observed from the soil sample of site II.

The total number of 11 VAM fungal species were isolated and identified from both the sites. *Glomus* dominated with seven species followed by *Acaulospora* with three species and *Gigaspora* with a single species. *Glomus fasciculatum* was found associated with all the eight genera of bryophytes including both the sites. Interestingly, one species *Glomus pustulatum* was found associated only with *Riccia* sp.\(^1\) at site I (Figure 1).

**Discussions**

The VAM fungi are mostly found in higher vascular plants because they have roots. It is obviously rare for thallophytes and bryophytes to have mycorrhiza, because they do not have true roots. However, Johnson (1977); Tarnau, Ronikier, and Unrug (1999); and Schubler (2000) reported occurrence of VAM fungi in bryophytes. In this study, we found that rhizospheric soil of collected genera of bryophytes harbour different VAM fungal species. Among these, VAM fungi belonging to the genus *Glomus* showed their dominance. This confirms their ubiquitous presence. The interesting thing is that some of the species of *Glomus* and *Acaulospora* were common in both the sites, but did not show host specificity. This suggests that neither local environmental conditions nor root exudation of host genera affect much on their occurrence. However, occurrence of *G. pustulatum* with *Riccia* sp.\(^2\) at site II is an exceptional case of host preference and site specificity. It is worth mentioning that these VAM fungal species not only occur in cold conditions, but also at high altitudes with these simple thaloid roots-less plants. *C. arafa*, *D. uckett*, and *L. ignone* (2003) reported that different patterns of arbuscular infections existed in liverworts. In *H. aplomitrium* and *T. trubia*, fungi enter intercellularly through plant mucilage secretion whereas in *M. archantiales*, they enter intracellularly through rhizoids (Lignone and Lopes 1989; Read, D. uckett, F. rancis, et al. 2000; Russell and Bulman 2005). Infection also differs in hornworts, where rhizoids are free of fungi (Schubler 2000). However, there were differences in the cytology of the infection zones. Notably, the upper arbuscular zone lies directly beneath the photosynthetic epidermal cells and is, therefore, close to the source of organic carbon. The invading endophyte possibly establishes and maintains infection by means of longitudinally growing hyphae in the lower zone, while active metabolic exchange occurs in the upper zones.

It may be deduced that cold and wet conditions do not restrict occurrence of *Glomus* and *Acaulospora*, but these conditions might be detrimental for other VAM fungi such as *G. gigaspora*, *Scelocystis*, *Scutellospora*, and *E. nirophospora*. Therefore, it is subject to further investigation.

**Acknowledgement**

Authors are thankful to Prof. S P Bajpai, Head, Department of Botany for providing laboratory facilities and to Prof. N K Soni for identifying the

---

**Figure 1** Distribution of VAM fungi in different bryophytes of Eastern and Western Himalayas

VAM – (vesicular arbuscular mycorrhizal); LMGC – *Glomus magnicaule*; Giga – *Gigaspora*; ASCB – *Acaulospora scrobiculata* (Trappe); ANCS – *A. nicolsonii* (Walker, Read, and Sanders); ASPN – *A. spinosa* (Walker); LFSC – *Glomus fasciculatum* (Thaxter Sensu Gerd.); LMSS – *Glomus moseae* (Nicol. and Gerd.); LOCT – *Glomus occultum* (Walker); LPST – *Glomus pustulatum* (Koske, Fries, Walker, and Dalpe); LDMR – *Glomus dimorphicum* (Boyetchko and Tewari); LABS – *Glomus ambisporum* (Smith and Schneck); LMSS – *Glomus magniculae* (Hall); Species code as per Perez and Schenck (1990).
bryophytes. Deepak Vyas and Mahendra K M ishra are thankful to the UGC (U niversity Grants C ommission) for the Research Award and their financial support. R K Gupta is thankful to the Principal of the Govt P G College, Rishikesh, Uttarakhand.

References

Subterranean gametophytic axes in the primitive liverworts Haplomitrium harbour a unique type of endophytic association with aseptate fungi
New Phytologist 160: 185–197

Cromwell B T. 1955
The alkaloid: a general introduction

Gerdemann J W and Nicolson T H. 1963
Spores of mycorrhizal endogone sp. extracted from soil by wet sieving and decanting
Transaction of the British Mycological Society 46: 235–244

Jackson M L. 1973
Soil Chemical Analysis
New Delhi: Prentice-Hall. pp. 239–241

Johnson P N. 1977
Mycorrhizal endogonaceae in a New Zealand forest
New Phytologist 76: 161–170

Kenrick P and Crane P R. 1977
The origin and early evolution of plants on land
Nature 269: 33–39

Ligrone R and Lopes C. 1989
Cytology and Development of mycorrhiza-like infection in the gametophyte of Conocephalum conicum (L.) Dum. (Marchantials, Hepatophyta)
New Phytologist 111: 423–433

Mishra R. 1968
Ecological Workbook
Calcutta: Oxford and IBH Publishing Company

Perez Y and Schenck N C. 1990
A unique code for each species of VA mycorrhizal fungi
Mycologia 52: 256–260

Piper C S. 1944
Soil and Plant Analysis
Australia: Hassel-Press

Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection
Transaction of the British Mycological Society 55: 158–161

Rabatin S C. 1980
The occurrence of the vesicular arbuscular mycorrhizal fungus Glomus tenuis with moss
Mycologia 72: 191–195

Symbiotic fungal association in lower land plants
Philosophical Transaction of the Royal society of London Series, Proceedings B 355: 815–831

Russell J and Bulman S. 2005
The liverworts Marchantia foliacea forms a specialized symbiosis with AM fungi in the genus Glomus
New Phytologist 165: 567–579

Schubler A. 2000
Glomus clonioideum forms an arbuscular mycorrhiza-like symbiosis with the hornwort Anthoceros punctatus
Mycorrhiza 10: 15–21

Turnau K, Ronikier M, and Unrug J. 1999
Role of mycorrhizal links between plants in establishment of liverwort thalli in natural habitats
Acta Societatis Botanicorum Poloniae 68: 63–68

Bibliography

Vesicular arbuscular mycorrhizal association with Lantana spp. in Sagar University Campus
Studies on arbuscular mycorrhizal fungi on *Maranta arundinacea* L. and associated plants in different soils of Kanyakumari district
P Charles*, S Kiruba**, V R Pinky*, S Israel Stalin*, and N R Laila Banu*

Introduction

AM (arbuscular mycorrhizae) are the most common endomycorrhizae. AMF (arbuscular mycorrhizal fungi) form symbiotic association with roots of most crop plants, wild herbaceous plants, and trees; as well as pteridophytes and some bryophytes. AM fungi have gained significance because of their role in soil fertility, nutrient uptake, biocontrol of plant diseases, and growth of plants used in afforestation (Bakshi 1974; Byra Reddy and Bhagyaraj 1988; T haper and K han 1973). Arrowroot (*Maranta arundinacea* L.) is an unexploited minor tuber crop cultivated for its starchy rhizome. It produces long, fleshy, and cylindrical rhizomes which contain very fine, easily digestible starch.

Arrowroot and its associated flora, along with symbiotic AM fungi, are recorded in this study. Arrowroot, in different soils, exhibits luxuriant growth of endomycorrhizae. Isolation and multiplication of efficient strains of AM F are important for better crop production as the rhizosphere of arrowroot and its associated plants harbour rich AM F flora. There is an immense scope for increasing the yield of arrowroot by adopting a suitable nutrient management schedule. Due to the hike in the cost of production of chemical fertilizers, the agricultural planners are compelled to reorient their approach towards cost-effective, cheap, and environmentally friendly source to supplement chemical fertilizers. Application of bio-fertilizers is the viable alternative. This study is carried out to identify native AM F in arrowroot rhizosphere and their efficacy on subsequent plant productivity.

Materials and methods

A survey was conducted to collect roots and rhizosphere soil samples of arrowroot and associated plants of three different soils in the Kanyakumari district (Table 1).

The soil samples collected were air dried, gently powdered, and sieved through a 2 mm sieve for analysis. The rhizosphere soils were used for isolating spores by wet sieving and decanting method (Gerdeman and Nicolson 1963). Attempts were made to detect the type of mycorrhizal association by examining root sections cleared by adopting the method given by Phillips and Hayman (1970).

Percentage root colonization was determined based on the number of root segments colonized by AM fungi. AM F isolates from various locations were identified with the help of the ‘Manual for the identification of VA mycorrhizal fungi’ by Schenck and Perez (1987). The physico-chemical characters – soil type, total nitrogen, available phosphorus, copper, and zinc are estimated following standard methods (Jackson 1967).

Results and discussion

Observations on arrowroot from different soils showed the association of AM F. The plants growing with arrowroot in red soil, sandy clay, and laterite soil differed markedly. The list of plants collected and their mycorrhizal status are given in Tables 2, 3, and 4.

Analysis of rhizosphere soils of arrowroot from different places revealed the presence of AM F spores. The spore abundance and diversity of AM F varied with location and soil types. The highest spore load was in laterite soil collected from Vilavancode, whereas the lowest in sandy clay soil was collected from Killiyoor. Observations of K ruckelman (1973); Kehri, Chandra, and M aheshwar (1987); and Lekha, Sivaprasad, Joseph,

---

*Department of Botany, **Department of Zoology, Scott Christian College (autonomous), Nagercoil - 629 003, India
Table 2  Incidence of AMF in *Maranta arundinacea* L. and associated plants in unit area (m²) of red soil

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Number of plants</th>
<th>VAM root colonization (%)</th>
<th>AMF species identified</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maranta arundinacea</em></td>
<td>12</td>
<td>61.5/12.71/11.42</td>
<td>Glomus mosseae,</td>
</tr>
<tr>
<td><em>Cardiospermum halicacabum</em></td>
<td>8</td>
<td>18.36/12.18/19.80</td>
<td>Glomus aggregatum,</td>
</tr>
<tr>
<td><em>Syndreella nodiflora</em></td>
<td>7</td>
<td>37.68/3.02/13.28</td>
<td>Acaulospora laevis,</td>
</tr>
<tr>
<td><em>Oldenlandia umbellata</em></td>
<td>10</td>
<td>17.96/9.26/12.5</td>
<td>Sclerocystis rubiformis,</td>
</tr>
<tr>
<td><em>Desmodium gangeticum</em></td>
<td>3</td>
<td>55.01/13.03/18.028</td>
<td>Glomus geosporum,</td>
</tr>
<tr>
<td><em>Amorphophalus campanulatus</em></td>
<td>3</td>
<td>24.42/12.98/26.42</td>
<td>Scutellospora sp,</td>
</tr>
<tr>
<td><em>Desmodium triflorum</em></td>
<td>7</td>
<td>9.22/7.18/0</td>
<td>Glomus constrictum,</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em></td>
<td>5</td>
<td>28.26/6.18/13.52</td>
<td>Glomus aggregatum</td>
</tr>
<tr>
<td><em>Phyllanthus amarus</em></td>
<td>4</td>
<td>78.94/12.18/19.72</td>
<td></td>
</tr>
<tr>
<td><em>Aerva lanata</em></td>
<td>12</td>
<td>55.0/11.59/0</td>
<td></td>
</tr>
<tr>
<td><em>Ageratum houstonianum</em></td>
<td>4</td>
<td>19.59/10.50/23.21</td>
<td></td>
</tr>
</tbody>
</table>

Spore number/100 g soil – 140.30

HC – Hypha; AC – Arbuscules; VC – Vesicles; AMF – arbuscular mycorrhizal fungi; VAM – vesicular arbuscular mycorrhiza

Table 3  Incidence of AMF in *Maranta arundinacea* and associated plants in unit area (m²) of sandy clay soil

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Number of plants</th>
<th>VAM root colonization (%)</th>
<th>AMF species identified</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maranta arundinacea</em></td>
<td>20</td>
<td>21.19/12.15/20.36</td>
<td>Glomus aggregatum,</td>
</tr>
<tr>
<td><em>Synedrella nodiflora</em></td>
<td>5</td>
<td>39.34/17.26/9.83</td>
<td>Glomus constrictum,</td>
</tr>
<tr>
<td><em>Euphorbia hirta</em></td>
<td>2</td>
<td>60.85/0/18.19</td>
<td>Sclerocystis rubiformis,</td>
</tr>
<tr>
<td><em>Centella asiatica</em></td>
<td>3</td>
<td>38.01/0/15.42</td>
<td>Glomus fasciculatum,</td>
</tr>
<tr>
<td><em>Isachne bourneorum</em></td>
<td>5</td>
<td>45.57/22.36/32.36</td>
<td>Glomus microcarpum,</td>
</tr>
<tr>
<td><em>Sida acuta</em></td>
<td>1</td>
<td>31.56/15.09/21.91</td>
<td>Glomus mosseae,</td>
</tr>
<tr>
<td><em>Leucas aspera</em></td>
<td>1</td>
<td>79.20/0/9.32</td>
<td></td>
</tr>
<tr>
<td><em>Oldenlandia umbellata</em></td>
<td>2</td>
<td>55.96/19.26/20.5</td>
<td></td>
</tr>
<tr>
<td><em>Phyllanthus amarus</em></td>
<td>1</td>
<td>27.88/12.15/57.12</td>
<td></td>
</tr>
<tr>
<td><em>Commelina sp.</em></td>
<td>1</td>
<td>18.16/0/0</td>
<td></td>
</tr>
<tr>
<td><em>Abutilon indicum</em></td>
<td>2</td>
<td>39.23/23.95/28.28</td>
<td></td>
</tr>
<tr>
<td><em>Spermacose hispida</em></td>
<td>2</td>
<td>50.51/41.94/12.89</td>
<td></td>
</tr>
<tr>
<td><em>Andrographis paniculata</em></td>
<td>2</td>
<td>35.79/17.26/19.72</td>
<td></td>
</tr>
</tbody>
</table>

Spore number/100 g soil – 108.50

HC – Hypha; AC – Arbuscules; VC – Vesicles; AMF – arbuscular mycorrhizal fungi; VAM – vesicular arbuscular mycorrhiza

Table 4  Incidence of AMF in *Maranta arundinacea* and associated plants in unit area (m²) of laterite soil

<table>
<thead>
<tr>
<th>Name of plant</th>
<th>Number of plants</th>
<th>VAM root colonization (%)</th>
<th>AMF species identified</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Maranta arundinacea</em></td>
<td>8</td>
<td>51.3/13.92/11.38</td>
<td>Glomus mosseae,</td>
</tr>
<tr>
<td><em>Moringa oleifera</em></td>
<td>1</td>
<td>45.27/15.18/9.83</td>
<td>Gigaspora rosea,</td>
</tr>
<tr>
<td><em>Syndreella nodiflora</em></td>
<td>5</td>
<td>84.52/17.82/20.86</td>
<td>Glomus reticulatum,</td>
</tr>
<tr>
<td><em>Galinsoga parviflora</em></td>
<td>3</td>
<td>62.36/0/25.17</td>
<td>Acaulospora laevis,</td>
</tr>
<tr>
<td><em>Carica papaya</em></td>
<td>2</td>
<td>33.26/0/5.03</td>
<td>Glomus geosporum,</td>
</tr>
<tr>
<td><em>Dioscorea alata</em></td>
<td>2</td>
<td>71.98/12.15/18.31</td>
<td>Glomus constrictum,</td>
</tr>
<tr>
<td><em>Thespesia populnea</em></td>
<td>1</td>
<td>53.73/27.38/22.15</td>
<td>Gigaspora aurigloba,</td>
</tr>
<tr>
<td><em>Amaranthus viridis</em></td>
<td>7</td>
<td>38.19/36.38/7.26</td>
<td>Acaulospora scrobiculata,</td>
</tr>
<tr>
<td><em>Acalypha indica</em></td>
<td>6</td>
<td>47.04/18.96/12.15</td>
<td>Glomus aggregatum,</td>
</tr>
<tr>
<td><em>Corchorus olitorious</em></td>
<td>2</td>
<td>22.15/27.32/0</td>
<td>Glomus microcarpum,</td>
</tr>
<tr>
<td><em>Ocimum tenuiflorum</em></td>
<td>2</td>
<td>68.94/0/12.18</td>
<td>Scutellospora calospora</td>
</tr>
<tr>
<td><em>Merrinia sp.</em></td>
<td>5</td>
<td>74.62/19.87/32.55</td>
<td></td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em></td>
<td>3</td>
<td>56.26/7.18/13.52</td>
<td></td>
</tr>
</tbody>
</table>

Spore number/100 g soil – 210.00

HC – Hypha; AC – Arbuscules; VC – Vesicles; AMF – arbuscular mycorrhizal fungi; VAM – vesicular arbuscular mycorrhiza

Mycorrhiza News **19**(4) • January 2008  21
et al. (1995) indicated the influence of soil type on incidence of spores and their population. The arrowroot rhizosphere soils showed obvious diversity of AMF population. Among the genera observed, Glomus was found to be dominating followed by Gigaspora, Acaulospora, Sclerocystis, and Scutellospora. Among the genera observed, Glomus sp, G. aggregatum occurred frequently in different soils. Predominance of Glomus over other genera of AMF, in Indian soils, was also reported by T Haper, K Amala, and Verma (1991); and Khalq and Janardhanan (1994).

The marked difference observed in the composition of AMF can be attributed to the influence of agro-climatic conditions and edaphic factors. Redhead (1977) and Koske (1987) found that nutritional conditions of the soil played an important role in deciding the richness of the species and population density of AM fungi. Soil conditions such as pH, temperature, texture, and others also govern the diversity of AM fungi (Land and Schonbeck 1991).

Mycorrhizal infection is characterized by intraradical and extrametrical hyphae, intracellular hyphal coils, inter or intracellular vesicles and arbuscules. Root colonization of 21.19%–61.50% was recorded in arrowroot collected from different soils. The highest and lowest percentage of hyphae was observed in root samples collected from Killiyoor and Vilavancode, respectively. Arbuscules and vesicles were observed in most of the root samples. Vesicles were dense in most of the samples observed. The highest percentage was seen in root samples collected from Killiyoor and the lowest in samples collected from Agasteeswaram. The relation between moisture content and incidence of microflora in root zones of crop plants was observed by Griffen (1963). High soil moisture or soil water potential reduces the infection of AMF by way of lowering oxygen tension of soils thereby hampering the chances of development of endophytes in the root region (Hayman 1983). AM fungi are very specific in their effect on plant species. They can vary in their effects on cultivars within a single plant species and can differ in their effect on plants in different ecosystems and soil types. The same isolate performing well in one agro-climatic region does not necessarily perform well in other regions. Thus, it becomes necessary to have a repository of these fungi from different agro-climatic regions for exploiting their agricultural potential.

References
Bakshi B K. 1974
Mycorrhizae and its role in forestry
Dehradun: Forest Research Institute. p. 89
[Project report PL 480]
Byra Reddy M S and Bhagyaraj D J. 1988
Selection of efficient VAM fungus for inoculating Leucaena in oxisols and vertisols
Gerden J W and Nicolson T H. 1963
Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting
Transactions of the British Mycological Society 46: 235–244
Griffin D N. 1963
Soil physical factors and the ecology of Fungi II. Behaviour of Pythium ultimum at small soil water suctions
Transactions of the British Mycological Society 46: 368–372
Hayman D S 1983
The physiology of vesicular-arbuscular endomycorrhizae symbiosis
Canadian Journal of Botany 61: 944–963
Jackson M L. 1967
Soil chemical analysis
New Delhi: Prentice Hall of India Pvt. Ltd
Kehri H K, Chandra S, and M aheswari S. 1987
Occurrence and intensity of VAM in weeds, ornamentals and cultivated plants in Allahabad and areas adjoining it
New Delhi: Jawaharlal Nehru University
Khalq A and Janardhanan K K. 1994
Variation of native VA mycorrhizal association on cultivated species of mint
Symbiosis 16: 75–82
Koske R E. 1987
Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient
Mycologia 79: 55–68
Kruckelman H W. 1973
Land S and Schonbeck F. 1991
Influence of different soil types on abundance and seasonal dynamics of vesicular-arbuscular mycorrhizal fungi in arable soils of North Germany
Mycorrhiza 1: 39–44
New approaches

A quick and precise technique for identifying ectomycorrhizas by PCR

A rapid procedure was developed by Mirco Iotti and Alessandra Zambonelli (2006) to amplify ITS (internal transcribed spacer) fragments directly from Tuber ectomycorrhizas, either synthesized in greenhouses or collected from fields (Mycological Research 110(1): 60–65, 2006). The addition of BSA (Bovine Serum Albumin) to the reaction mixtures overcame the presence of reaction inhibitors present in fungal and root cells, and enabled the amplification of ITS regions directly from ectomycorrhizal tissues. This method is cheaper and less time consuming than conventional procedures, and reduces the time required from 1–4 hours to a few minutes. It is also much more sensitive, allowing the identification of a small fragment of mycorrhizal root tip. Because of this, it is possible to select only the target fungal tissue and hence minimize the risk of contamination by saprobic or other mycorrhizal species. This method also avoids the use of toxic or hazardous substances, and could have a wider application in other areas of applied mycology.
Introduction

Biological control of plant pathogens is currently a key practice in sustainable agriculture because it is based on the management of a natural resource, that is, certain rhizosphere organisms that are common components of ecosystems and are known to develop antagonistic activities against the harmful organisms including plant parasitic nematodes. Root-knot nematodes (Meloidogyne spp.) are the most economically important PPN (plant parasitic nematode) group worldwide (Perez and Lewis 2004). Out of all the PPN, Meloidogyne spp. is reported to be the most damaging nematode on vegetable crops. Amongst the vegetable crops, tomato has been found to be most easily affected and prone to huge economic damage by root-knot nematode (Minton and Baujard 1990). Management of nematode infestations on this crop has mainly been through chemical means. However, this approach of chemical control through nematicides and its indiscriminate use affects soil health and is therefore prohibited in organic cultivation. EPN (entomopathogenic nematodes) appears to be a promising and safe alternative to nematicides due to their ability to control plant parasitic nematodes like root-knot nematode Meloidogyne spp., Belanolaimus spp., Criconemella, and others, in different crops (Bird and Bird 1986; Shibashi and Kondo 1986; Lewis, Grewal, and Sardanelli 2001; Grewal, Lewis, and Gaugler 1996; Grewal, Martin, Miller, et al. 1997; Perez and Lewis 2002, 2004). The AM (arbuscular mycorrhizal) fungi have also been found to exhibit a suppressive effect on the root-knot development and reproduction on various crops. The present work deals with more accurate understanding on the synergism or compatibility of mycorrhizal association with EPN. It will help in developing strategies for controlling root-knot disease of tomato and open up avenues for effective integrated disease management practices.

Materials and methods

A greenhouse experiment was conducted at TERI, Gual Pahari, Gurgaon. Pot experiment was carried in sterilized potting mixture to understand the interactive development of AM fungi in presence of EPN. There were four treatments of different concentration (0, 1000, 2000, and 3000 ij [infective juveniles]) of EPN (Heterorhabdits sp13.31) with mycorrhized tomato plants. Treatments were replicated four times. Larval juveniles were inoculated to tomato plants of AM fungi (Glomus intraradices). Plants were harvested after eight weeks of transplantation and analysed for the different growth, nutrient, and mycorrhizal parameters following standard protocols.

Nutrient Analyses:

Shoots of all plant species were severed just above earth level, weighed, dried at 70 °C for 48 h and weighed again. For nutrient analysis, plant material was ground to pass through a 0.5 mm screen and digested in a H₂SO₄−H₂O₂ mixture. The P (phosphorus) and N (nitrogen) contents in the digest were determined as described by Jackson (1973).

Mycorrhizal parameters

Spore count

Wet sieving and decanting method was used for spore count (Gerdemann and Nicolson 1963).

Estimation of percent colonization using frequency distribution method

Percent AM fungus colonization in roots was determined on 100 root segments (1 cm each) per
sample. Roots were stained as reported by Phillips and Hayman (1970). Root pieces were mounted on glass slides and examined under 400× magnification with the help of a compound microscope. Colonization was determined according to the method of Biermann and Linderman (1981) and expressed as the percent of each root segment length that was colonized.

Acid/alkaline phosphatase activity in hypha (modified azo dye method using fast blue RR salt)
In alkaline phosphatase, 0.05 M tris-citric acid buffer (pH 9.2) was used, whereas in acid phosphatase, 50 mM (millimolar) acetate buffer (pH 4.0) was used. Dissolved 0.02 g fast blue RR salt, 0.02 g a napthyl acid phosphate, 0.86 ml of M gCl₂ (0.5 mg/ml), and 1.28 ml of M nCl₂·4H₂O (0.5 mg/ml) in 20 ml of 0.05 M tris-citric acid buffer (pH 9.2) for alkaline phosphatase activity and in 20 ml of 0.05 M acetate buffer (pH 4.0) for acid phosphatase activity.

The hyphae were incubated in this reaction medium for 24 h at 25 °C and washed with sterile distilled water to remove the reaction medium. The hyphae were then mounted in lacto glycerol. Observations were taken randomly for 50 fields (using ocular micrometer for hyphal length) black point per unit length of mycelium recorded as active length percentage of total mycelium scanned.

Statistical analysis
Treatment effects were determined by one-way ANOVA using a completely randomized design. Significant differences between treatments were confirmed by DMRT (Duncan’s Multiple Range Test) using Co-stat Statistical Software (Cohort, Berkeley, Calif.).

Results and discussion
The biomass and root length of tomato plant was not significantly affected with EPN inoculation (Table 1). The plant shoot P and N content was not affected by the inoculation of EPN (Table 2). Fallon, Kaya, Gaugler et al. (2002) also reported that application of Steinernema riobrave (EPN) in tomato plants had no effect on leaf number and plant biomass. The overall study indicates that the nutrient content of plant is not affected by the presence of EPN.

Mycorrhiza spore number, colonization, and acid and alkaline phosphatase activity was not significantly affected by EPN inoculation (Figure 1). The results indicate mycorrhiza and EPN-compatible existence in rhizospheric environment. EPN appear to be a promising and safe alternative to

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Biomass (gm)</th>
<th>Root Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPN 0 ij + AMF</td>
<td>12.78 a</td>
<td>88.91 a</td>
</tr>
<tr>
<td>EPN 1000 ij + AMF</td>
<td>12.68 a</td>
<td>86.31 a</td>
</tr>
<tr>
<td>EPN 2000 ij + AMF</td>
<td>12.44 a</td>
<td>87.77 a</td>
</tr>
<tr>
<td>EPN 3000 ij + AMF</td>
<td>12.41 a</td>
<td>87.51 a</td>
</tr>
<tr>
<td>LSD (p&lt;0.05)</td>
<td>0.694</td>
<td>3.597</td>
</tr>
</tbody>
</table>

AMF – arbuscular mycorrhizal fungi; EPN – entomopathogenic nematodes; ij – infective juveniles; LSD – least significant difference [The values are the mean of four replicates. Values with different alphabets in column indicate significant difference using the Duncan’s Multiple Range Test at p<0.05]
nematicides due to their ability to control plant parasitic nematodes like root-knot nematode *Meloidogyne* sp., *Belanolaimus* sp. *Crenonemella*, and others in different crops. AM fungi have also been found to exhibit a suppressive effect on the root-knot development and reproduction on various crops (Sharma, Bhargava, Verma, et al. 1994; Azcon and Barea 1996). Both mycorrhizal fungi as well as root-knot nematode are commonly found inhabiting the rhizosphere and colonizing the roots of a host plant. These two groups of microbes exert positive and opposite effects on plant growth. EPN also share the same rhizospheric environment. This EPN species and AM fungi interaction study showed their compatibility in the rhizospheric environment. EPN and root-knot nematodes respond to the secondary metabolites released by the plant root systems into the rhizosphere (Grewal, Lewis, and Gaugler 1996). The EPN–AM synergism may be a promising approach for the control of root-knot diseases as the biological control of root-knot nematode by AM fungi may further be enhanced by EPN. Further research is needed to elucidate the relationship between EPN and root-knot nematodes and this could eventually utilized as an integrated tool for managing the plant parasitic root-knot nematode.

References

Azcon-Aguilar C and Barea J M. 1996
Arbuscular mycorrhizal and the biological control of soil-borne plant pathogens - an overview of the mechanisms involved
*Mycorrhiza* 6: 457-464

Biermann B and Linderman R. 1981
Quantifying vesicular-arbuscular mycorrhizae: proposed method towards standardization
*New Phytologist* 87: 63-67

Bird A F and Bird J. 1986
Observations on the use of insect parasitic nematodes as means of biological control of root-knot nematodes
*International Journal for Parasitology* 16: 511-516

Fallon D J, Kaya H K, Gaugler, R and Sipes B. 2002
Effects of entomopathogenic nematodes on *Meloidogyne javanica* on tomato and Soybean
*Journal of Nematology* 34(3): 239-245

Gerdemann J W and Nicolson T H. 1963
Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting
*Transactions of British Mycological Society* 46: 235-244

Grewal P, Lewis E E, and Gaugler R. 1996
Response of infective stage parasites (Rhabditida: Steiner nematidae) to volatile cues from infected hosts
*Journal of Chemical Ecology* 23: 503-515

Suppression of plant-parasitic nematode populations in turf grass by application of entomopathogenic nematodes
*Biocontrol Science and Technology* 7: 393-399

Ishibashi N and Kondo E. 1986
*Steinernema feltiae* (DD-136) and *Steinernema glaseri*: persistence in soil and bark compost and their influence on native nematodes
*Journal of Nematology* 38: 310-316

Jackson M L. 1973
*Soil Chemical Analysis*
New Jersey: Prentice-Hall, Englewood Cliffs

Lewis E E, Grewal P S, and Sardanelli S. 2001
Interactions between the *Steinernema feltiae*-Xenorhabdus bovienii insect pathogen complex and the root-knot nematode *Meloidogyne incognita*
*Biological Control* 21: 56-62

Minton N A and Baujard P. 1990
*Nematode parasites of peanuts*
UK: CAB International

Perez E E and Lewis E E. 2002
Use of entomopathogenic nematodes to suppress *Meloidogyne incognita* on greenhouse tomatoes
*Journal of Nematology* 34: 171-174

Perez E E and Lewis E E. 2004
Suppression of *Meloidogyne incognita* and *Meloidogyne hapla* with entomopathogenic nematodes on greenhouse peanuts and tomatoes
*Biological Control* 30: 336-341

Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection
*Transactions of British Mycological Society* 55: 158-161

*Interaction between the endomycorrhizal fungus Glomus fasciculatum and the root-knot nematode, Meloidogyne incognita* on tomato
*Indian Journal of Nematology* 24(2): 34-39
Recent references

The latest additions to the network’s database on mycorrhiza are published here for the members’ information.

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:

- Annals of Botany
- Annals of Microbiology
- Biochemistry
- Biological Reviews
- Canadian Journal of Plant Science
- Current Opinion in Plant Biology
- Forest Ecology and Management
- Ecology
- M invia Biotecnologica
- Mycorrhiza
- New Phytologist
- Plant Biology
- Plant and Soil
- Soil Biology and Biochemistry
- South African Journal of Botany
- Plant Physiology and Biochemistry
- Phytopathologist
- Plant Biology

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

<table>
<thead>
<tr>
<th>Name of the author(s) and year of publication</th>
<th>Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)</th>
</tr>
</thead>
</table>
| Alberton O*, Kuyper T W, and Gorissen A. 2007 | **Competition for nitrogen between Pinus sylvestris and ectomycorrhizal fungi generates potential for negative feedback under elevated CO₂**
Plant and Soil **296**(1-2): 159-172
[Alberton O, University of Wageningen and Research Center, Department of Soil Quality POB 47, NL-6700 AA Wageningen, the Netherlands] |
| Aldrich-Wolfe L. 2007 | **Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community**
Ecology **88**(3): 559-566.  
[Aldrich-Wolfe L, Kansas State University, Division of Biology, Ackert Hall, Manhattan, KS 66506] |
| Akiyama K. 2007 | **Chemical identification and functional analysis of apocarotenoids involved in the development of arbuscular mycorrhizal symbiosis**
Bioscience Biotechnology and Biochemistry **71**(6): 1405-1414
[Akiyama K, University of Osaka Prefecture, Grad School Life and Environmental Science, Naka K u, 1-1 Gakuuencho, Sakai, Osaka 5998531, Japan] |
| Anderson I C* and Cairney J W G. 2007 | **Ectomycorrhizal fungi: exploring the mycelial frontier**
Fems Microbiology Reviews **31**(4): 388-406
[Anderson I C, Macaulay Land Use Research Institute, Aberdeen AB15 8QH, Scotland] |
[Garcia-Romera I, CSIC, Estac Expt Zaidin, Department of Microbiology Suelo and Sistemas Simbiot, Prof Albareda 1 Apdo 419, E-18008 Granada, Spain] |
| Arriagada C A, Herrera M A, and Ocampo J A*. 2007 | **Beneficial effect of saprobe and arbuscular mycorrhizal fungi on growth of Eucalyptus globulus co-cultured with Glycine max in soil contaminated with heavy metals**
[Ocampo J A, CSIC, Estac Expt Zaidin, Department of Microbiology Suelo and Sistemas Simbiot, Profesor Albareda 1, E-18008 Granada, Spain] |
<table>
<thead>
<tr>
<th>Name of the author(s) and year of publication</th>
<th>Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)</th>
</tr>
</thead>
</table>
| Aucina A, Rudawaska M *, Leski T, Skridaila A, Riepsas E, Iwanski M. 2007 | Growth and mycorrhizal community structure of Pinus sylvestris seedlings following the addition of forest litter  
Applied and Environmental Microbiology 73(15): 4867-4873  
[Rudawaska M, Polish Academy of Science, Institute of Dendrol, 5 Parkowa Str, PL-62035 Kornik, Poland] |
| Audet P and Charest C *. 2007 | Dynamics of arbuscular mycorrhizal symbiosis in heavy metal phytoremediation: meta-analytical and conceptual perspectives  
Environmental Pollution 147(3): 609-614  
[Charest C, Univ Ottawa, Department of Biology, Ottawa Carleton Institute of Biology, 30 Marie Curie St, Ottawa, ON K1N 6N5, Canada] |
Mycorrhiza 17(3): 185-193  
[Baptista P, ESAB, CIM O, Apartado 1172, P-5301855 Braganca, Portugal] |
Mycorrhiza 17(5): 355-373  
[Barroetavena C, CIEFAP, Centre Invest Forestal, CC 14, RA-9200 Esquel, Chubut, Argentina] |
| Barros L, Baptista P, Ferreira I C F R*. 2006 | Influence of the culture medium and pH on the growth of saprobic and ectomycorrhizal mushroom mycelia  
Minerva Biotecnologica 18(4): 165-170  
[Ferreira I C F R, Inst Politecn Braganca, CIMO Escola Superior Agrária, Campus Santa Apolonia, Apartado 1172, P-5301855 Braganca, Portugal] |
| Bennett A E* and Bever J D. 2007 | Mycorrhizal species differentially alter plant growth and response to herbivory  
Ecology 88(1): 210-218  
[Bennett A E, University of Calif Davis, 4348 Storer Hall, Davis, CA 95616] |
| Bergero R*, Lanfranco L, Ghignone S, Bonfante P. 2007 | Enhanced activity of the Gmar MT1 promoter from the mycorrhizal fungus Gigaspora margarita at limited carbon supply  
Fungal Genetics and Biology 44(9): 877-885  
[Bergero R, University of Edinburgh, Institute of Evolutionary Biology, W Mian Road, Edinburgh EH9 3JT, Midlothian, Scotland] |
| Brearley F Q*, Scholes J D, Press M C, Palfner G. 2007 | How does light and phosphorus fertilisation affect the growth and ectomycorrhizal community of two contrasting dipterocarp species?  
Plant Ecology 192(2): 237-249  
[Brearley FQ, M anchester Metropolitan University, Department of Environmental and Geographical Science, Chester St, Manchester M 1 5GD, Lancs, England] |
| Buee M*, Courty P E, Mignot D, Garbaye J. 2007 | Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community  
[Buee M, INRA, UM R1136 Interact Arbes Microorganisms, F-54280 Seichamps, France] |
| Cameron D D*, Johnson I, Leake J R, Read D J. 2007 | Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid Goodyera repens  
Annals of Botany 99(5): 831-834  
[Cameron D, University of Sheffield, Department of Animal and Plant Science, Alfred Denny Bldg, Western Bank, Sheffield S10 2TN, S Yorkshire, England] |
Title of the article, name of the journal, volume number, issue number, page numbers
(address of the first author or of the corresponding author, marked with an asterisk)

Screening and selecting arbuscular mycorrhizal fungi for inoculating micropropagated apple rootstocks in acid soils
Plant Cell Tissue and Organ Culture 90(2): 117-129
[de Mendonca, M M, Federal University of Santa Catarina, Department of Microbiology and Parasitology, Cx P 476, BR-88040900 Florianopolis, SC, Brazil]

Structural investigation of a heteroglycan isolated from the fruit bodies of an ectomycorrhizal fungus Astraeus hygrometricus
Carbohydrate Research 342(7): 982-987
[Islam S S, Vidyasagar University, Department of Chemistry and Chemical Technology, W Midnapore 721102, W Bengal, India]

The arbuscular mycorrhizal fungus Glomus mosseae gives contradictory effects on phosphorus and arsenic acquisition by Medicago sativa Linn
Science of The Total Environment 379(2-3): 226-234
[Zhu Y G, Chinese Academy of Science, Department of Soil Environmental Science, State Key Laboratory and Environmental Chemistry and Ecotoxicology, Ecoenvironmental Science Research Centre, Beijing 100085, People's R China]

Plant nitrogen acquisition and interactions under elevated carbon dioxide: impact of endophytes and mycorrhizae
Global Change Biology 13(6): 1238-1249
[Hu S J, North Carolina State University, Department of Plant Pathology, Box 7616, Raleigh, NC 27695]

Chen X H, Zhao B*. 2007
Arbuscular mycorrhizal fungi mediated uptake of lanthanum in Chinese milk vetch (Astragalus sinicus L.)
Chemosphere 68(8): 1548-1555.
[Zhao B, Huazhong Agriculture University, State Key Laboratory of Agricultural Microbiology, Wuhan 430070, Peoples R China]

Chen Y L, Liu S, and Dell B. 2007
Mycorrhizal status of Eucalyptus plantations in south China and implications for management
Mycorrhiza. 17(6): 527-535
[Chen Y L, University of Western Australia, School of Plant Biology, Perth, WA 6009, Australia]

The characteristics of rhizosphere microbes associated with plants in arsenic-contaminated soils from cattle dip sites
Science of The Total Environment 378(3): 331-342
[Zhang R, University of Wollongong, School of Biological Sciences, Wollongong, NSW 2522, Australia]

Cline E*, Vinyard B, and Edmonds R. 2007
Spatial effects of retention trees on mycorrhizas and biomass of Douglas-fir seedlings
[Cline E, University of Washington, Box 358436, Tacoma, WA 98402]

Influence of arbuscular mycorrhizal fungi on growth and essential oil composition in Ocimum basilicum var. Genovese
Caryologia 60(1-2): 106-110
[Berta, G., University of Piemonte Orientale, DI SAV, Via Bellini 25-G, I-15100 Alessandria, Italy]
<table>
<thead>
<tr>
<th>Name of the author(s) and year of publication</th>
<th>Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>de la Noval B, Perez E, Martinez B, Leon O, Martinez-Gallardo N, Delano-Frier J*. 2007</td>
<td><strong>Exogenous systemin has a contrasting effect on disease resistance in mycorrhizal tomato (Solanum lycopersicum) plants infected with necrotrophic or hemibiotrophic pathogens</strong>&lt;br&gt;Mycorrhiza <strong>17</strong>(5): 449–460&lt;br&gt;[Delano-Frier J, Unidad Biotecnol and Ing Genet Plantas Cinvestav, Campus Guanajuato, Km 9-6 Libramiento Norte Carret, Irapuato 36500, Gto, Mexico]</td>
</tr>
<tr>
<td>Grebenc T* and Kraigher H. 2007</td>
<td><strong>Types of ectomycorrhiza of mature beech and spruce at ozone-fumigated and control forest plots</strong>&lt;br&gt;Environmental Monitoring and Assessment <strong>128</strong>(1–3): 47–59&lt;br&gt;[Grebenc T, Slovenian Forestry Institute, Vecna Pot 2, Ljubljana 1000, Slovenia]</td>
</tr>
</tbody>
</table>
### Forthcoming events
#### Conferences, congresses, seminars, symposia, and workshops

<table>
<thead>
<tr>
<th>Event</th>
<th>Date</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Dr H C Uwe Jens Nagel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agricultural Extension and Communication Sciences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Humboldt Universität zu Berlin, Luisenstraße 53, 10099 Berlin, Germany</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel. +49 (30) 2093 6510</td>
<td>E-mail uj.nagel(at) agrar.hu-berlin.de</td>
<td></td>
</tr>
<tr>
<td>Fax +49 (30) 2093 6512</td>
<td>Website <a href="http://www.mace-events.org/mace/mace.html">www.mace-events.org/mace/mace.html</a></td>
<td></td>
</tr>
<tr>
<td><strong>World Sustainable Development Forum: Sustainable Development and Climate Change</strong></td>
<td>7–9 February 2008</td>
<td>New Delhi, India</td>
</tr>
<tr>
<td>The Summit Secretariat, TERI, D arbari Seth Block</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I H C Complex, Lodhi Road, New Delhi -110 003, India</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel. +91 11 24682100/41504900</td>
<td>E-mail <a href="mailto:dsds@teri.res.in">dsds@teri.res.in</a></td>
<td></td>
</tr>
<tr>
<td>Fax +91 11 24682144/24682145</td>
<td>Website <a href="http://www.teriin.org/dsds">http://www.teriin.org/dsds</a></td>
<td></td>
</tr>
<tr>
<td>Registration <a href="http://www.teriin.org/dsds/2008/registration08.htm">http://www.teriin.org/dsds/2008/registration08.htm</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Biotechnology: State of the Art and Prospects of Development and International Exhibition ’Biotech World’</strong></td>
<td>11–13 March 2008</td>
<td>Moscow, Russia</td>
</tr>
<tr>
<td>JSC “EXPO-BIOCHIM - TECHNOLOGIES”</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moscow International Congress</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moscow, 117218, B. Cheremushkinskaya St., 34, of. 552</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tel. +7 (495) 981-70-51, 981-70-54</td>
<td>E-mail <a href="mailto:aleshnikova@mosbiotechworld.ru">aleshnikova@mosbiotechworld.ru</a></td>
<td></td>
</tr>
<tr>
<td>Fax +7 (495) 981-70-51, 981-70-54</td>
<td>Website <a href="http://www.mosbiotechworld.ru/eng/expo.php">www.mosbiotechworld.ru/eng/expo.php</a></td>
<td></td>
</tr>
<tr>
<td><strong>Plant-Microbial Interactions 2008 (PMI-2008)</strong></td>
<td>2–6 July 2008</td>
<td>Kraków, Poland</td>
</tr>
<tr>
<td>Katarzyna Turnau</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conference venue: Strict City Centre</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-mail <a href="mailto:pmi2008@eko.uj.edu.pl">pmi2008@eko.uj.edu.pl</a></td>
<td>Website <a href="http://www.eko.uj.edu.pl/mycorrhiza/pmi/">http://www.eko.uj.edu.pl/mycorrhiza/pmi/</a></td>
<td></td>
</tr>
<tr>
<td><strong>ICOM 2008: International Congress on Membranes and Membrane Processes</strong></td>
<td>12–18 July 2008</td>
<td>Honolulu, Hawaii, USA</td>
</tr>
<tr>
<td>The North American Membrane Society</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICOM 2008 Secretariat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University of Texas at Austin, Center for Energy and Environmental Resources 10100 Burnet Road, Building 133-R 7100, Austin, TX 78758</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Website <a href="http://www.membranes.org">http://www.membranes.org</a></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ICPP 2008: 9th International Congress of Plant Pathology</strong></td>
<td>24–29 August 2008</td>
<td>Torino, Italy</td>
</tr>
<tr>
<td>Valentina Communication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Via Cibrario 27, 10143 Torino, Italy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fax +39 0114374318</td>
<td>E-mail <a href="mailto:info@icpp2008.org">info@icpp2008.org</a></td>
<td></td>
</tr>
<tr>
<td>Website <a href="http://www.icpp2008.org">http://www.icpp2008.org</a></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Mycorrhiza News 19(4) • January 2008*
**HARVEST MORE**

**VAM - For the first time in the world**
Produced and processed through Sterile Technology

**Mycorrhiza (Fungus root) is the symbiotic association between plant roots and soil fungus. The Endomycorrhiza (VAM) is an obligate symbiont. VAM (Vesicular Arbuscular Mycorrhiza) for Agriculture, Forestry and Horticulture crops is being produced through the most advanced technology, is now commercially available for farming applications.**

The Mass production technology of VAM has been developed by TERI-DBT for the first time in the world. We are the first in the world to produce VAM in commercial scale using the sterile technology of TERI-DBT.

**Named as ECORRHIZA-VAM (Powder form) & NURSERRHIZA-VAM (Tablet form), the biotic root inoculant gives the following benefits to the crop plants.**

- Increased phosphorus uptake
- Increased micronutrient uptake
- Enhanced water uptake
- Increased resistance to pathogens and pests
- Enhanced tolerance to soil stress viz. high salt levels, heavy metal toxicity, drought, high temperatures etc.
- Enhanced transplant survival
- Enhanced beneficial microbial population in the root zone.

**BIO-FERTILIZERS**

We are also producing the Bio-fertilizers for Nitrogen fixation (Azospirillum, Azotobacter), Phosphate solubilization (Bacillus megaterium var.phosphaticum).

1. **AZOSPIRILLUM**: This is an associative symbiotic Nitrogen fixing bacteria. It fixes the atmospheric Nitrogen through the action of Nitrogenase enzyme. It secretes auxin, cytokinin & gibberellin like substances which promote the growth of plants. It is useful for non-leguminous crops.

**DOSAGE**: 4 kgs. per acre

2. **AZOTOBACTER**: This is a free living, Non-symbiotic Nitrogen fixing bacteria. It fixes atmospheric Nitrogen through the action of Nitrogenase enzyme. It is specially recommended for Paddy crop.

**DOSAGE**: 4kgs. per acre

3. **BACILLUS MEGATERIUM VAR. PHOSPHATICUM (PHOSPHOBACTER)**: This bacteria solubilizes the unavailable phosphorus in the soil through the secretion of weak organic acids such as formic, acetic, lactic acids etc. These acids reduce the pH and bring about the dissolution of bound forms of phosphate. Some of the hydroxy acids may chelate with Calcium and Iron resulting in effective solubilization and utilization of phosphorus.

**DOSAGE**: 4 kgs. per acre

*For further details contact:*

K.C. SUGAR & INDUSTRIES CORPORATION LIMITED, VUYYURU-521 165.

Phone: 0867-23400, Fax: 0867-232640, E-mail: vjwkc@vymd@sancharnet.in

**Editor** Alok Adholeya • **Associate Editor** T P Sankar • **Assistant Editor** Richa Sharma

Printed and published by Dr R K Pachauri on behalf of The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi - 110 003, and printed at Multiplexus (India), C-440, DsIDC, Narela Industrial Park, Narela, Delhi - 110 040.