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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

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Phenolic compounds impart resistance to plants against pathogenic organisms. Mycorrhizal 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 *Sessamum 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. Histochemical 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 these amides was performed and their NMR and MS data coincided with those of the native compounds. 14C labelled precursors were used for incorporation experiments to evaluate their biosynthetic rate in endomycorrhized and control onion roots. Time course studies were done with endomycorrhized and control onion and leek roots prior to quantification of their free and wall-bound amides. A significant increase of these compounds was observed in the cell walls of endomycorrhized roots of both plant species. Such compounds are known to exhibit antiviral activity and decrease cell wall digestibility. Increased levels of cinnamoyl amides in endomycorrhized roots may confer resistance of these roots to soil pathogens (Grandmaison, Furlan, Ola'h, *et al*. 1990).

Studies conducted at the Plant Biochemistry Laboratory, Department of Biology, Concordia University, SGW Campus, De Maisonneuve Boulevard West, Montreal, Quebec, Canada, on phytochemical characterization of the major phenolic compounds and their ultrastructural localization carried out on onion (*Allium cepa*) roots colonized by *Glomus intraradix* and *Glomus versiforme* showed that ferulic and P-coumaric acids, as well as N-feruloyltyramine were the major phenolic metabolites bound to the cells walls of VAM onion roots. The results from mycorrhized and control plants suggested the presence of a mechanism leading to the oxidative condensation of phenols, the latter process depending on the presence or absence of symbiosis. Bioassays reveal that N-feruloyltyramine induced the branching of hyphae and reduced total fungal development. The overall results suggested that the progressive binding of phenolic compounds in VAM roots was directly involved in the control of VAM endophytic establishment and development, as it gradually reduced the plasticity and elasticity of the symbiotic matrix. Phenolic compounds bound to the cell walls could also be indirectly responsible for the resistance of VAM roots to pathogenic fungi, since they resulted in increased resistance by the cell wall to the action of digestive enzymes (Grandmaison, Olah, Calsteren, *et al*. 1993).

In studies conducted at the United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Centre, 600 East Mermaid Lane, Philadelphia, USA, root cultures of carrot (an ubiquitous host for VAM) and sugar beet (a non-host plant) were used to compare the constitutive phenolic compounds associated with the cell wall or present in the cytoplasm of both hosts. Phenolic acids were released from purified cell walls by alkaline hydrolysis and were separated and identified by HPLC, TLC (thin layer chromatography) and UV (ultra violet) absorption spectra analyses. Two phenolic acids unique to carrot root cell walls were identified as p-HBA (p-hydroxybenzoic acid) and vanillic acid. Sugar beetroot cell walls had ferulic acid as a major constituent and contained several unique phenylpropanoids, which were not identified. Caffeic acid was found only in the cytoplasm of carrot roots and was present in the conjugated form (chlorogenic acid). The sugar beet cytoplasm also contained several identified hydroxycinnamic acid-type phenolics, which were

not found in carrot roots (Nagahashi, Abney, and Doner 1996).

Effect of ectomycorrhizal infection on phenolic compounds

In studies conducted at the Institut fur Botanik, Spezielle Botanik, Mykologie, Eberhard-Karls-Universitat, Tubingen, Morgenstelle, Tubingen, Germany, p-HBA glucoside, picein, piceatonnol and its glucoside, isorhapontin, 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-HBA 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 (Munzenberger, Heilemann, Strack, *et al.* 1990).

Similar studies conducted at the Institut fur Wald und Forstokologie, Dr. Zinn-Weg, O-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 larch 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 amethystea* contained none of the identified phenolics. The growth of the mycorrhizal fungi, *L. amethystea* 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 (Munzenberger 1993; Munzenberger, Kottke, and Oberwinkler 1995).

In studies conducted at the Department of Botany, University of Hong Kong, Pokfulam Road, Hong Kong, polyphenols histochemically detected in fresh uninfected roots of *Quercus*, *Castanopsis,* and *Lithocarpus* – growing in Hong Kong and shown to be condensed tannins – were found mainly as intracellular material in the cells of the root cap, the epidermal layer, and the endodermis. 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 xylopyrus* 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, Alexandria, 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 + $CaCl_2$]) 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 Universita di Torino, Viale Matholi, Torino, Italia, on the activity of cell-wall-bound

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. Ultrastructural 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 *Nicotiana tabacco* and VAM fungus, *Glomus 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 roots These data indicate that the first reaction of the root cells to the invasion of VAM is a defence response (Blilou, 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, USA, 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 phenoloxidase activity were developed by adding specific precursors such as l-dopa, otolidine or spring aldazine 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 *Casatnea sativa* and *Quercus robur* to a symbiont, *Pisolithus tinctorius*. 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 $CaCl_2$ (calcium chloride) and SDS (sodium dodecyl sulphate) was higher on mycorrhized plants (roots and leaves). The ratio between the activities after activation by $CaCl_2$ 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 United States Department of Agriculture, Forest Service, Southern Forest Experiment Station, 2500 Shreveport Highway, Pineville, LA, USA, on container-grown shortleaf pine (*Pinus echinata*) seedlings inoculated or not with *P. tinctorius* at germination, and both fertilized semi-weekly with a modified Hoagland's solution with zero or 0.4 mM boric acid, showed that inoculation with *P. tinctorius* increased the root phenolic concentration of 16- and 24-week-old seedlings. Inoculation with *P. tinctorius* also increased root phenol oxidase activity in 12-, 16-, and 24-weeks old seedlings. The fertilizer treatment with boric acid reduced the phenolic concentration of *P. tinctorius* ectomycorrhizae after 24 weeks, although boric acid fertilizer did not affect the phenol oxidase activity of *P. tinctorius* grown in vitro and 12-week-old uninoculated roots. Boric acid fertilizer thus influenced the phenolic relations of the ectomycorrhizal association, possibly through a boric acid-induced increase in phenol oxidase activity (Sword and Garrett 1994).

In 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 intracellular and occurred in majority of the isolates. The strong laccase reaction was predominantly 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 *Morchella* 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 fungi grown asymbiotically can release extracellular enzymes capable of oxidizing a wide range of aromatic compounds (Gramss, Gunther, and Fritsche 1998).

Further studies conducted at the above Institute on ectomycorrhizal fungi, *Suillus granulatus* and *Paxillus involutus*, grown in both LC (liquid culture) and in symbiosis with *Pinus sylvestris* seedlings under sterile conditions, LC 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. LC 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 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-blotting 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 PSYP1 cDNA which was most abundantly expressed in short roots. PSYP1 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 PSYP1 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 PSYP1 belonged to the group of secreted class-III peroxidases. The presence of 10 tyrosine residues and putative auxin-binding regions in PSYP1 suggested that the function of the enzyme was associated with cell-wall formation in short roots. The down regulation of PSYP1 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, Nyman, Kalkkinen, *et al*. 2001).

Studies were conducted at the University of Sydney (Nepean), Mycorrhiza Research Group, School of Science, 10, Kingswood, NSW, 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_2O_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 (Wyss and Wiemken 1988; Wyss, 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 Leguminosae (Fabaceae), 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 plant 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 phytoalelxin 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 latter phytoalexin-specific genes (Dixon, Harrison, and Paiva 1995).

Studies conducted at the Department of Microbiology, School of Biology, Madurai Kamraj University, Madurai, Tamil Nadu, India, on the accumulation of phytolalexin in cowpea during the infection of *Glomus fasciculatum*, *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, Ubalthoose, *et al.* 1988; Gunasekaran, Ubalthoose, Sundaresan, *et al.* 1988).

Production of antifungal compounds by ecto-mycorrhizal fungi

In studies conducted at the Centre de Recherche en Biologie Forestiere, Faculte de Foresterie et de Geomatique, Universite Laval, Sainte-Foy, Quebec,

Canada, two antifungal compounds isolated from the liquid culture medium of *Pisolithus arhizus* were identified as p-hydroxybenzoylformic acid and (R)-(-)-p-hydroxymandelic acid and given the trivial names pisolithin A and pisolithin B, respectively. The efficacy of the compounds to inhibit conidial germination of *T. hartigii* was compared with that of commercially available structural analogues. A comparable range of effectiveness for 50% germination inhibition (GI50) of conidia was recorded. The commercially available synthetic compounds (R)-mandelic acid, benzoylformic acid, and recemic p-hydroxymandelic acid had GI50 values of 82, 72, and 59 microg/ml respectively, as compared with the natural compounds pisolithin A (67 microg/ml) and pisolithin B (71 microg/ml). Two synthetic S enantiomers of mandelic acid, (S)-mandelic acid and (S)-(+)-p-hydroxymandelic acid were the most effective compounds, with GI50 values of 31 and 33 microg/ml respectively. A sodium salt of mandelic acid had no activity below 500 microg/ml. Pisolithin A and pisolithin B were compared with polyxin D for inhibition of hyphal growth, as measured by protein estimation. Both pysolithin A and B measured higher levels of putative extractable protein than polyxin D, but less mycelial-wet weight was measured. It was suggested that the pisolithins caused a disruption of cell turgor. A measurement of mycelial dry weights of phytopathogens, incubated with the commercially available analogues benzoylformic acid and racemic p-hydroxymandelic acid, indicated that benzoylformic acid was either more effective than, or as effective as racemic p-hydroxymandelic acid or nystatin in arresting fungal growth. For ectomycorrhizal fungi confronted with the pisolithins, both compounds were effective in inhibiting the growth of an equal number of fungi, but benzoylformic acid caused a growth stimulation of some ectomycorrhizal fungi (Kope, Tsantrizos, Fortin, *et al*. 1991).

In another study conducted at the Department of Chemistry, McGill University, Montreal, Quebec, Que.H3A 2K6, Canada, the antibiotic compounds, p-hydroxybenzoylformic acid (2-(4'-hydroxyphenyl)-2-oxoethanoic acid, pisolithin A) and (R)-(-)-o-hydroxymandelic acid, ((R)-(-)-2-(4'-hydroxyphenyl)-2-hydroxyethanioc acid, pisolithin B) were isolated from the growth culture of *P. tinctorius*. Both of these metabolites, and a few structurally related compounds, were shown to inhibit spore germination and cause hyphal lysis to the phytopathogenic fungi, *R. solani*, *Verticillium dahliae*, *Pyrenochaete terrestris*, *Pythium* spp., *Phytophthora* sp. and *Fusarium solani,* and the dermatogenic fungi, *Microsporum gypseum* and *Trichophyton equinum*. The mode of action of the metabolites was speculated that chelation of metal ions may be involved (Tsantrizos, Kope, Fortin, *et al.* 1991).

In further studies conducted at the above University, the antifungal antibiotics from Pisolithin B (p-hydroxymandelic acid), 2-(4'-hydroxyphenyl)-2-hydroxyethanoic acid, isolated from *P. tinctorius* was shown to have the absolute (R) configuration. The stereochemistry 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, Universita, L'Aquila, Italy, on the effects of Tuber 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 *Aspergillus alliaceus*). 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-Methyl 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*, *Rhaphanus raphanistrum*) had detectable antifungal compounds in extracts from roots. Two species from the Chenopodiaceae (*Spinacea oleracea*, *Beta vulgaris*) and one species from Amaranthaceae (*Amaranthus 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 (*Lactuaca 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 produced by the action of myrosinase on glucosinalbin. The data provided evidence of a possible role for glucosinolates in determining the non-mycotrophic status in *B. kaber* (Schreiner and Koide 1993).

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Research finding papers

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

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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 MUB (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 $CaCl_2$ 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-nitro phenol 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-nitro phenol, 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.

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

Plant species	*Phosphatase activity*				*Root infection (%)*	*VAM spore population density/100 g soil*
	Acid phosphatase		*Alkaline phosphatase*			
	Inoculated(µg)	*Uninoculated*(µg)	*Inoculated*(µg)	*Uninoculated*(µg)		
R. serpentina	188.0	74.0	78.0	67.0	60	380
C. forskohlii	46.0	29.5	46.5	26.5	50	345

VAM – vesicular arbuscular mycorrhizal; *C. forskohlii – Coleus forskohlii; R. serpentina – Rauvolfia serpentina*

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.

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Response of Rangpur lime to different endomycorrhizal species for germination, growth parameters, and graft success

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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* (*Elevusine 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 a 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 (3.81 mm and 3.94 mm, respectively) (Table 1). At 180 DAS, the highest root length was recorded in *G. leptotichum* and *G. mosseae* inoculated rootstocks (30.77 cm and 29.17 cm, respectively) as compared to other treatments. At 270 DAS, the highest primary root length was recorded in rootstock treated with *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*

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

Treatments	*Germination* (%)	*Rootstock height* (cm)		*Number of leaves*		*Rootstock diameter* (mm)		*Primary root length* (cm)		*Number of lateral roots*	
		180 DAS	*270 DAS*	*180 DAS*	*270 DAS*	*180 DAS*	*270 DAS*	*180 DAS*	*270 DAS*	*180 DAS*	*270 DAS*
T_1 – Control	78.67	9.03	12.17	17.00	21.27	2.28	3.81	18.92	24.60	38.33	68.00
T_2 – *Acaulospora laevis*	87.20	16.57	21.27	29.80	45.27	3.95	4.64	26.40	30.30	62.33	87.00
T_3 – *Glomus mosseae*	90.00	16.07	19.92	30.53	47.73	3.56	4.84	29.17	34.20	68.00	99.00
T_4 – *Glomus bagyaraji*	86.00	15.29	18.63	25.85	30.73	2.47	3.94	15.17	28.70	37.33	72.00
T_5 – *Glomus leptotichum*	98.69	15.56	19.76	28.00	41.62	3.72	4.32	30.77	39.80	76.00	120.00
S.Em±	1.57	0.95	0.93	1.86	2.58	0.07	0.08	0.78	0.33	1.70	6.26
C.D. at 5%	4.72	2.86	2.80	5.62	7.79	0.22	0.25	2.36	0.986	5.13	18.8

DAS – days after sowing

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

Treatments	*Nitrogen* (%)				*Protein* (%)			
	180 DAS		*270 DAS*		*180 DAS*		*270 DAS*	
	Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
T_1 – Control	0.84	0.49	0.42	0.21	5.26	3.16	2.63	1.31
T_2 – *Acaulospora laevis*	1.89	1.75	1.05	0.77	11.83	10.90	6.56	4.81
T_3 – *Glomus mosseae*	3.01	2.59	2.24	1.12	19.29	16.28	14.00	7.00
T_4 – *Glomus bagyaraji*	2.31	2.03	0.91	0.56	1.45	12.66	5.69	3.50
T_5 – *Glomus leptotichum*	1.61	0.91	1.12	0.84	10.06	5.70	7.00	5.25
S.Em±	0.06	0.02	0.03	0.02	0.23	0.09	0.17	0.13
C.D. at 5%	0.17	0.06	0.08	0.06	0.68	0.26	0.51	0.39

DAS – days after sowing

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* (99.01) 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 3 Effect of arbuscular mycorrhizal fungi on percent root colonization and spore count of Rangpur lime rootstocks

Treatments	*Root colonization* (%)	*Spore count* (number of chlamydospores/ 50 g soil)	*Graft success* (%)	*Graft survival* (%)
T_1 – Control	37.50	92.25	61.09	68.18
T_2 – *Acaulospora laevis*	92.50	942.25	85.00	94.12
T_3 – *Glomus mosseae*	95.50	966.25	90.00	97.22
T_4 – *Glomus bagyaraji*	87.50	853.25	81.25	84.62
T_5 – *Glomus leptotichum*	90.50	937.25	95.35	97.56
S.Em±	0.65	2.63	0.73	0.73
C.D. at 5%	1.94	7.91	2.09	2.09

(Simon 1984). 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 4 Correlation studies of microbiological parameters versus graft success and graft survival of Rangpur lime rootstocks treated with arbuscular mycorrhizal fungi

Root Colonization (%)	*Spore count* (number of chlamydospores/ 50 g of soil)	*Graft success* (%)	*Graft survival* (%)
1.000	0.999*	0.927*	0.939*
	1.000	0.939*	0.947*
		1.000	0.976*
			1.000

*Significant at P = 0.01 level

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Vesicular arbuscular mycorrhizal association in Bryophytes isolated from Eastern and Western Himalayas

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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 glomelean fungi that form arbuscules in their thalli. Fine endophytes (glomelean 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 Mishra (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 Mishra (1968). Per cent organic carbon was measured by the method of Piper (1944).

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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 Jungermanials 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* sp[1] 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 Hepaticopsida. A larger number of VAM fungal spores was recorded in *Riccia* sp.[2]; that is 1244/100 g of dry soil, followed by 1169 in *Plageochasma* sp. and 1112 in *Marchantia* sp. and least 810 were recorded with *Astrella* sp. Maximum 60% colonization was also observed with *Riccia* sp.[2] and minimum 40% colonization was recorded with *Astrella* sp. *Marchantia* shows 55% and *Plageochasma* shows 50% colonization. Similarly maximum seven VAM species was found with *Riccia* sp.[1] followed by six sp. in *Marchantia* sp. Four and two VAM sp. were recorded in *Plageochasma* and *Astrella*, respectively. As mentioned earlier, that arbuscules and vesicles were observed in *Marchantia* 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.

Name of Bryophytes	*Colonization* (%)	*Spore population/ 100 g soil*	*Presence of fungal strains*			*Number of VAM species*	*Occurrence of VAM fungal species*
			Mycelium	*Arbuscules*	*Vesicles*		
Riccia sp.	65	1120	+	–	–	5	ANCS, ASCB, LFSC, LMSS, LMGC
Pellia sp.	*75*	*1289*	+	+	+	*8*	ASCB, ASPN, ANCS, LFSC, LMSS, *LABS, LDMR, GABD*
Notothyllus sp.	54	1092	+	–	–	5	ASPN, LABS, LOCT, LFSC, LMSS
Funaria sp.	45	960	+	–	–	3	LFSC, LABS, LMCG
Riccia sp.	60	1244	+	–	–	7	ASPN, ASCB, LFSC, LMSS, LOCT, LPST, LDMR
Marchantia sp.	55	1162	+	+	+	6	ASCB, ANCS, LFSC, LMCG, LABS, LMSS
Plagiochasma sp.	50	1129	+	–	–	4	ASPN, LFSC, LOCT, GABD.
Asterella sp.	40	810	+	–	–	2	LFSC, LMSS

VAM – vesicular arbuscular mycorrhizal; LMGC – *Glomus magnicaule* (Hall); GABD – *Gigaspora albida* (Schenck and Smith); ASCB – *Acaulospora scorbiculata* (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

Site	*pH*	*Conductance*	*N* (kg/h)	*P* (kg/h)	*K* (kg/h)	*C* (%)	*Base deficiency*	*Colour*	*Texture*	*Odour*
Site I Darjeeling	7.8	0.30	296	10.4	325	4.4	+	Brown	Loamy	Volatile
Site II Musoorie	6.5	0.24	360	8.0	198	2.4	++	Gray	Sandy	Ammonium

+ – 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 fasiculatum* was found associated with all the eight genera of bryophytes including both the sites. Interestingly, one species *Glomus pustullosum* was found associated only with *Riccia* sp[1] at site I (Figure 1).

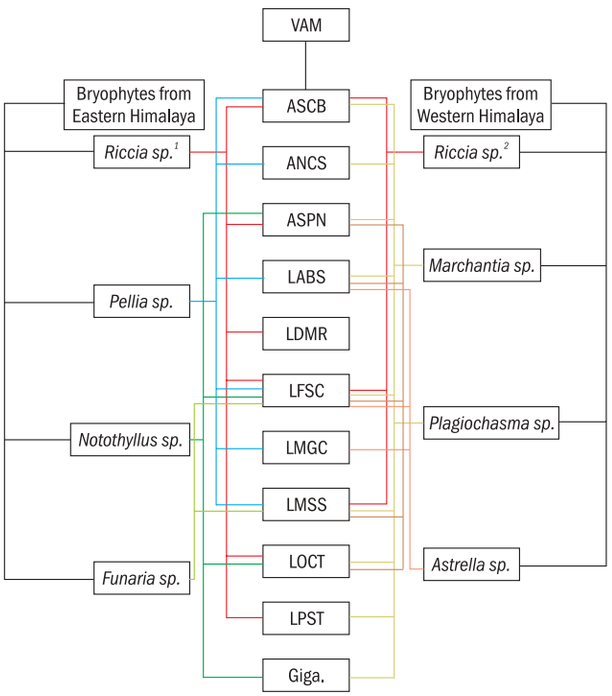

Figure 1 Distribution of VAM fungi in different bryophytes of Eastern and Western Himalayas

VAM – (vesicular arbuscular mycorrhizal); LMGC – *Glomus magnicaule*; Giga – Gigaspora; ASCB – *Acaulospora scorbiculata* (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).

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. pustullatum* 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 thalloid roots-less plants. Carafa, Duckett, and Ligrone (2003) reported that different patterns of arbuscular infections existed in liverworts. In *Haplomitrium* and *Treubia*, fungi enter intercellularly through plant mucilage secretion whereas in Marchantiales, they enter intracellularly through rhizoids (Ligrone and Lopes 1989; Read, Duckett, Francis, *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 *Gigaspora*, *Sclerocystis*, *Scutellospora*, and *Entrophospora*. Therefore, it is subject to further investigation.

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

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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; Thaper and Khan 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 AMF are important for better crop production as the rhizosphere of arrowroot and its associated plants harbour rich AMF 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 AMF 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. AMF 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).

Table 1 Sampling locations and physico-chemical characteristics of the rhizosphere soils of *Maranta arundinacea* and associated plants

Factor	*Agasteeswaram*	*Killiyoor*	*Vilavancode*
Soil type	Red soil	Sandy clay	Laterite
Soil pH	5.9	6.8	6.4
Moisture (%)	8.0	8.5	9
Organic matter (%)	0.85	0.95	0.96
Available nitrogen (g/kg)	0.340	0.380	0.102
Available phosphorus (g/kg)	0.004	0.015	0.003
Available potassium (g/kg)	0.196	0.210	0.095
Copper (ppm)	0.28	0.21	0.14
Zinc (ppm)	0.82	0.66	0.50
Manganese (ppm)	0.93	1.51	2.64
Iron (ppm)	3.8	4.1	3.62

ppm – parts per million

Results and discussion

Observations on arrowroot from different soils showed the association of AMF. 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 AMF spores. The spore abundance and diversity of AMF 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 Kruckelman (1973); Kehri, Chandra, and Maheswari (1987); and Lekha, Sivaprasad, Joseph,

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Table 2 Incidence of AMF in *Maranta arundinacea* L. and associated plants in unit area (m^2) of red soil

Name of plant	Number of plants	VAM root colonization (%)			AMF species identified
		HC	AC	VC	
Maranta arundinacea	12	61.5	12.71	11.42	*Glomus mosseae,*
Cardiospermum halicacabum	8	18.36	12.18	19.80	*Glomus aggregatum,*
Synedrella nodiflora	7	37.68	3.02	13.28	*Acaulospora laevis,*
Oldenlandia umbellata	10	17.96	9.26	12.5	*Sclerocystis rubiformis,*
Desmodium gangeticum	3	55.01	13.03	18.028	*Glomus geosporum,*
Amorphophalus campanulatus	3	24.42	12.98	26.42	*Scutellospora sp,*
Desmodium triflorum	7	9.22	7.18	0	*Glomus constrictum,*
Boerhaavia diffusa	5	28.26	6.18	13.52	*Glomus aggregatum*
Phyllanthus amarus	4	78.94	12.18	19.72	
Aerva lanata	12	55.0	11.59	0	
Ageratum houstonianum	4	19.59	10.50	23.21	
Axonopus compressus	7	62.50	20.15	58.25	

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 (m^2) of sandy clay soil

Name of plant	Number of plants	VAM root colonization (%)			AMF species identified
		HC	AC	VC	
Maranta arundinacea	20	21.19	12.15	20.36	*Glomus aggregatum,*
Synedrella nodiflora	5	39.34	17.26	9.83	*Glomus constrictum,*
Euphorbia hirta	2	60.85	0	18.19	*Sclerocystis rubiformis,*
Centella asiatica	3	38.01	0	15.42	*Glomus fasciculatum,*
Isachne bourneorum	5	45.57	22.36	32.36	*Glomus microcarpum,*
Sida acuta	1	31.56	15.09	21.91	*Glomus mosseae*
Leucas aspera	1	79.20	0	9.32	
Oldenlandia umbellata	2	55.96	19.26	20.5	
Phyllanthus amarus	1	27.88	12.15	57.12	
Commelina sp.	1	18.16	0	0	
Abutilon indicum	2	39.29	23.95	28.28	
Spermacose hispida	2	50.51	41.94	12.89	
Andrographis paniculata	2	35.79	17.26	19.72	

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 (m^2) of laterite soil

Name of plant	Number of plants	VAM root colonization (%)			AMF species identified
		HC	AC	VC	
Maranta arundinacea	8	51.3	13.92	11.38	*Glomus mosseae,*
Moringa oleifera	1	45.27	15.18	9.83	*Gigaspora rosea,*
Synedrella nodiflora	5	84.52	17.82	20.86	*Glomus reticulatum,*
Galinsoga parviflora	3	62.36	0	25.17	*Acaulospora laevis,*
Carica papaya	2	33.26	0	5.03	*Glomus geosporum,*
Dioscorea alata	2	71.98	12.15	18.31	*Glomus constrictum,*
Thespesia populnea	1	53.73	27.38	22.15	*Gigaspora aurigloba,*
Amaranthus viridis	7	38.19	36.38	7.26	*Acaulospora scrobiculata,*
Acalypha indica	6	47.04	18.96	12.15	*Glomus aggregatum,*
Corchorus olitorious	2	22.15	27.32	0	*Glomus microcarpum,*
Ocimum tunuiflorum	2	68.94	0	12.18	*Scutellospora calospora*
Merrimia sp	5	74.82	19.87	32.55	
Boerhaavia diffusa	3	56.26	7.18	13.52	

Spore number/100 g soil – 210.00

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

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. Identification of AMF cultures revealed the presence of five genera comprising twenty species. Among the genera observed**,** *Glomus* was found to be dominating followed by *Gigaspora, Acaulospora, Sclerocystis, and Scutellospora*. Among the *Glomus* sp, *G. aggregatum* occurred frequently in different soils. Predominance of *Glomus* over other genera of AMF, in Indian soils, was also reported by Thaper, Kamala, and Verma (1991); and Khaliq 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 Griffin (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.

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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.

Effect of entomopathogenic nematode on arbuscular mycorrhizal fungi inoculated tomato plant's growth, mycorrhizal colonization, and enzyme activity of hyphae

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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; Ishibashi 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 H_2SO_4–H_2O_2 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 per cent 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 $MgCl_2$ (0.5 mg/ml), and 1.28 ml of $MnCl_2.4H_2O$ (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 1 Biomass (dry weight) and root length of tomato plant in presence of AMF and EPN

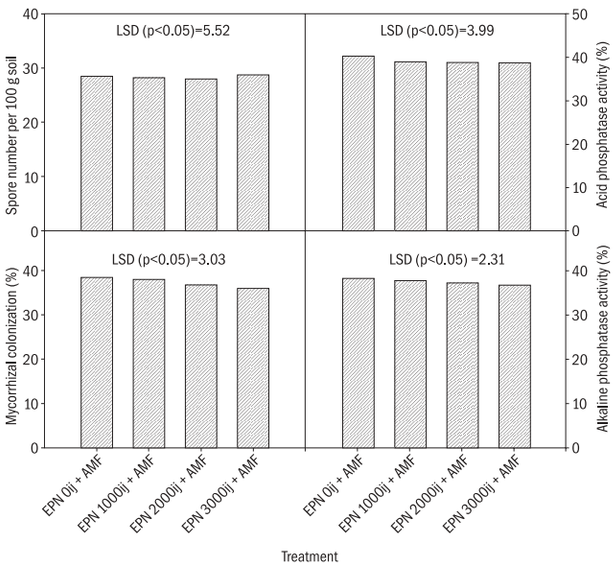
Treatment	*Biomass* (gm)	*Root Length* (cm)
EPN 0 ij + AMF	12.78 a	88.91 a
EPN 1000 ij + AMF	12.68 a	86.31 a
EPN 2000 ij + AMF	12.44 a	87.77 a
EPN 3000 ij + AMF	12.41 a	87.51 a
LSD ($p \leq 0.05$)	0.694	3.597

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 $p \leq 0.05$]

Table 2 Shoot phosphorus and nitrogen of tomato plant in presence of AMF and EPN

Treatment	*Phosphorus* (%)	*Nitrogen* (%)
EPN 0 ij + AMF	0.326 a	2.32 a
EPN 1000 ij + AMF	0.317 a	2.19 a
EPN 2000 ij + AMF	0.309 a	2.09 a
EPN 3000 ij + AMF	0.288 a	1.94 a
LSD ($p \leq 0.05$)	0.046	0.414

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 $p \leq 0.05$]



AMF – arbuscular mycorrhizal fungi; EPN – entomopathogenic nematodes; ij – infective juveniles; LSD – least significant difference

Figure 1 Spore number and mycorrhizal colonization of tomato plants and ACP and ALP activity of hypha in presence of EPN
[The values are the mean of four replicates. Bars with different alphabets indicate significant difference using the Duncan's Multiple Range Test at $p \leq 0.05$].

nematicides due to their ability to control plant parasitic nematodes like root-knot nematode *Meloidogyne* sp., *Belanolaimus* sp. *Criconemella,* 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.

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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*
- *Minerva Biotecnologica*
- *Mycorrhiza*
- *New Phytologist*
- *Plant Biology*
- *Plant and Soil*
- *Soil Biology and Biochemistry*
- *South African Journal of Botany*
- *Plant Physiology and Biochemistry*
- *Phytologist*
- *Plant Biology*

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

Name of the author(s) and year of publication	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)
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 CO_2** *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]
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