Vol. 19 No. 3
October 2007

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 .

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.

Contents

Biochemical changes in mycorrhizal synthesis

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

Establishment of mycorrhizal symbiosis is a complex developmental process that comprises a multistep cascade of events. This requires that complex morphogenetic, biochemical, physiological, and molecular changes occur in both fungus and roots. The most pronounced change that occurs in ectomycorrhizal synthesis is differentiation of new anatomical features (swollen root apex, hyphal mantle, Hartig net, and so on). However, many subtle biochemical changes also take place after inoculation. It is also likely that the vegetative growth of ectomycorrhizal hyphae and root tissues, and their ability to switch to symbiotic organ formation are basically controlled by symbiotic genes or at least a tightly coordinated read-off of genetic signals between both partners (Hilbert and Martin 1990). This write-up deals with such varied types of biochemical changes that accompany mycorrhizal symbiosis in plants.

Morphological and biochemical changes in mycorrhizal synthesis

In ectomycorrhizal synthesis

Studies conducted at the Centre de Recherches Forestieres de Nancy, INRA (Institut National de la Recherche Agronomique), Champenoux 54280 Seichamps, France, on protein biosynthesis in roots during the early stages of ectomycorrhiza formation on *Eucalyptus globulus* sub sp. *bicostata* using compatible and incompatible isolates of *Pisolithus tinctorius* showed that there were changes in polypeptide composition within hours, following contact of the compatible mycelium with the roots, well before the differentiation of typical symbiotic tissues. At this stage, at least seven symbiosis-related proteins (ectomycorrhizins) accumulated in root tissues.

In vivo incorporation of (^{35}S) methionine by ectomycorrhizas, followed by electrophoresis of the labelled proteins, revealed that most of these differences in polypeptide concentrations, including the ectomycorrhizin accumulation, were the result of differential protein biosynthesis rather than post-translation modifications of the polypeptides. The results indicate a functional developmental role for these proteins (Hilbert, Costa, and Martin 1991).

In another study conducted at the above-mentioned institute, cell fractions enriched in endoplasmic reticulum and plasma membranes were isolated from the roots of *E. globulus*, from free-living mycelium of *P. tinctorius*, and from *E. globulus–P. tinctorius* ectomycorrhizas. The effect of mycorrhiza formation on polypeptide levels was examined by 2D (two-dimensional) PAGE (polyacrylamide gel electrophoresis). The membrane fractions of each tissue had distinctive polypeptide patterns on 2D gels. Silver-stained gels showed that the differentiation of mycorrhizas caused increases and decreases in a number of polypeptides, but no unique membrane polypeptides (ectomycorrhizins) were induced by mycorrhiza development. Fluorographs of 2D gels of the microsomal membranes isolated from free-living

partners and ectomycorrhizal roots labelled with (^{35}S) methionine in vivo also showed that mycorrhiza formation induced changes in the synthesis of a number of polypeptides. Molecular turnover of three fungal membrane glycoproteins with high molecular weights, which were isolated and purified to homogeneity by concanavalin-A-sepharose affinity chromatography, was measured during the development of *E. globulus–P. tinctorius* mycorrhizas (Henrion and Martin 1990).

In further studies conducted at the above-mentioned institute, PAGE of ^{35}S-labelled proteins was used to examine the changes in protein biosynthesis during the early stages of differentiation of *P. tinctorius* ectomycorrhizas of *Eucalyptus grandis*. Three distinct isolates of *P. tinctorius* were chosen based on the rate of ectomycorrhizal formation (infectivity) with *E. grandis*. Isolate H-506 was not able to induce mycorrhiza. Isolate 441 showed moderate infectivity and isolate H2144 exhibited a high infectivity. Mycorrhizae were produced in vitro in a system where seeds were germinated in the presence of fungal mycelium and exudates. The non-mycorrhizal isolate caused no changes in root protein biosynthesis, whereas the other two isolates caused major changes in protein biosynthesis. During mycorrhizal development, there was a marked inhibition of plant polypeptide synthesis, enhanced accumulation of some fungal polypeptides, and emergence of symbiosis-specific polypeptiedes (Burgess, Laurent, Dell, et al. 1995).

In further studies conducted at the institute, spatial and temporal expressions in the *Eucalyptus* spp.–*P. tinctorius* symbiosis were characterized by cell fractionation, 2D PAGE, and immunochemical assays. Biochemical investigations revealed drastic alterations experienced by fungal cell wall polypeptides during the early stages of the ectomycorrhizal interaction. These included increased synthesis of 32-kDa acidic polypeptides, together with a decrease in the content of a major 95-kDa mannoprotein, gp95 (Tagu and Martin 1996).

In studies conducted at the Laboratoire de Phytonique, Universite d'Angers, 2 Bd Lavoisier, 49045 Angers, Cedex, France, polypeptide changes at different stages of mycorrhiza development were analysed in birch (*Betula pendula*) as preliminary step towards elucidation of the molecular basis of ectomycorrhiza differentiation. Time sequencing of the stages in the infection process of clonal plants inoculated with a compatible isolate of *Paxillus involutus* revealed that by eight days, mature ectomycorrhizas were obtained. Total phenol-extracted proteins of roots during the mycorrhiza formation stage (two to eight days post-inoculation) were separated by 2D PAGE, and the resulting patterns compared with non-mycorrhizal roots and mycelium. Alteration in the concentration of polypeptides from the host plant roots was limited even after eight days of contact between the symbionts. However, seven novel polypeptides were detected four days after inoculation, three of them already present in two-day-old ectomycorrhizas. These findings demonstrate that symbiosis-related polypeptides accumulate in ectomycorrhizal roots before any of the morphological changes characterizing the symbiotic state take place (Simoneau, Viemont, Moreau, et al. 1993).

In vesicular arbuscular mycorrhizal fungi

In studies conducted at the Estacion Experimental del Zaidin, Prof. Albareda 1, 18008 Granada, Spain, changes in gene expression in onion roots during the establishment of VAM (vesicular arbuscular mycorrhizal) symbiosis with *Glomus mosseae* were studied. Polypeptides were obtained by in vitro translation of total root RNA extracted from VAM-colonized and non-colonized root tissue of onion (cv. Babosa), and resolved by 2D PAGE. VA (vesicular arbuscular) mycorrhization led to the appearance of eight new polypeptides, and the disappearance of seven polypeptides in VAM-colonized roots. It was thus concluded that gene expression is altered in response to morphological and physiological changes from the establishment of VA mycorrhizas (Garcia-Garrido, Toro, and Ocampo 1993).

Studies were conducted at the Laboratoire de Phytoparasitologie INRA/CNRS, Station de Genetique et d'Amelioration des Plantes, INRA, Dijon Cedex, France, on changes in gene expression during the establishment of AM (arbuscular mycorrhizal) symbiosis in tobacco roots from an amphidiploid hybrid, *Nicotiana glutinosa – Nicotiana debneyi*. Polypeptide patterns from control roots and from roots infected by *G. mosseae* or *Glomus intraradices* were resolved by 2D PAGE and followed in a time-course analysis. AM infection led to significant modifications in polypeptide patterns with (i) decreased amounts of some polypeptides, (ii) increased accumulation of others, and (iii) appearance of newly induced polypeptides. Comparisons made during infection development by the two *Glomus* species demonstrated that protein modifications changed in relation to the mycorrhizal state of the tobacco roots (Dumas-Gaudot, Guillaume, Tahiri-Alaoui, et al. 1994).

In earlier studies conducted at the above-mentioned laboratory, soluble protein profiles were compared in non-mycorrhizal roots of tobacco and roots infected with different endomycorrhizal fungi in

order to investigate changes in translation events during endomycorrhizal establishment. Extracts of soluble proteins were analysed by PAGE on slab gels. The presence of certain proteins (β-proteins), typically synthesized during host–microbe interactions, was detected using β-protein antibody by ELISA (enzyme-linked immunosorbant assay) and by immunoblotting after transfer of PAGE-separated proteins to nitrocellulose. Synthesis of this type of host proteins, together with reported enhanced phytoalexin production during VA endomycorrhizal formation, shows that certain similar modifications in host physiology can occur in both symbiotic and pathogenic fungal interactions (Dumas, Goamomazzo-Pearson, and Gianinazzi 1988).

In further studies at the laboratory, changes following inoculation of pea roots (wild type cv. Frisson) (myc^+, nod^+) with the AM fungus, *G. mosseae*, were analysed by 2D PAGE. A different polypeptide pattern was obtained in a mycorrhiza-resistant pea genotype P2 (myc^-, nod^-) inoculated with *G. mosseae*. In order to further characterize the polypeptide modifications detected and to provide evidence for some possible symbiosis-related proteins, a time-course experiment; from appressoria formation to fully developed symbiosis; was carried out on two genotypes allowing fungal colonization: the wild type and its isogenic mutant P56 (myc^+, nod^-). The same experiment was done with the mycorrhiza-resistant pea genotype (myc^-, nod^-). After *G. mosseae* inoculation, 12 additional polypeptides were characterized in the two mycorrhiza-compatible pea genotypes, which were never observed in root extracts from the mycorrhiza-resistant mutant. Five polypeptides were first detected in the early stage of the symbiosis (five days after inoculation). The induction and accumulation of these polypeptides seem to be more correlated to the establishment of the functional symbiosis than to the recognition stages and appresorium formation. Furthermore, none of the additional polypeptides were detected in the mycorrhiza-resistant pea genotype. This mutant was more characterized by a great repression of polypeptides. In addition, upregulated and downregulated polypeptides from the mycorrhiza-compatible genotypes were different from those of the mycorrhiza-resistant genotype (Samra, Dumas-Gaudpt, and Gianinazzi 1997).

In further studies conducted at the institute, protein extracts of tomato roots colonized with the AM fungus, *G. mosseae*, were analysed by 2D gel electrophoresis in order to investigate the synthesis of AM-related polypeptides. Polypeptide patterns of mycorrhizal tomato roots were compared not only to those of non-infected ones, but also to those of tomato roots infected with the pathogenic fungus, *Phytophthora parasitica*. Comparisons of various polypeptide profiles showed that additional polypeptides were induced specifically in response to AM symbiosis versus pathogenic infection. Moreover, comparison of polypeptide patterns of *G. mosseae* colonized and *P. parasitica* infected tomato roots with those of either germinated spores of the AM fungus or of axenically grown pathogenic fungus suggested that some of the additional polypeptides were of plant origin (Dassi, Samra, Dumas-Gaudot, *et al.* 1999).

Ectomycorrhiza-specific polypeptides

In studies conducted at the Laboratoire de Microbiologie, Institut National de la Recherche Agronomique Champenoux, Seichamps, France, the polypeptide contents of *Eucalyptus–Pisolithus* mycorrhizas, uninfected *E. globulus* short roots, and free-living *P. tinctorius* mycelia were compared in an effort to investigate the presence of any symbiosis-specific genes that are expressed specifically during the development of ectomycorrhizas. Analysis of the soluble proteins by 2D PAGE showed that most of the polypeptides observed in mycorrhizas were already synthesized in free-living mycelia. The effects of mycorrhizal infection on polypeptide accumulation fell into three distinct categories: (i) decreased amounts of a large number of polypeptides, (ii) increased accumulation of a comparatively few polypeptides, and (iii) appearance of ectomycorrhizins (mycorrhiza-specific polypeptides). Approximately, 50% of the fungal polypeptides and more than 80%–90% of the plant polypeptides resolved on gels disappeared during the development of ectomycorrhizas. Among 500–520 polypeptides, 6–10 polypeptides, called ectomycorrhizins, were ectomycorrhiza specific because they were present in ectomycorrhizas, but not in free-living mycelia or in non-infected roots. The results showed differential accumulation of ectomycorrhizins during mycorrhiza development (Hilbert and Martin 1988 a, b).

Another study at the above-mentioned institute on the analysis of soluble and membrane proteins by SDS (sodium dodecyl sulphate) and 2D PAGE in *Eucalyptus–Pisolithus* ectomycorrhizas showed that most of the polypeptides observed in mycorrhizas were already synthesized in uninfected roots or in free-living mycelia. The level of about 30% of the fungal polypeptides and 50% of the plant polypeptides resolved on gels was regulated during ectomycorrhizal development. Most of the changes detected involved a decrease or an increase in the concentration of

proteins synthesized in free-living mycelia or root cells. Four polypeptides were present in ectomycorrhizas but not in free-living mycelia or uninfected short roots. These polypeptides for which the name ectomycorrhizins is suggested, thus appear to be ectomycorrhiza specific (Hilbert and Martin 1988c).

In another similar study at the above-mentioned institute, the polypeptide content of *Eucalyptus–Paxillus* ectomycorrhizas, uninfected *E. globulus* roots, and free-living *P. involutus* mycelia was similarly compared in an effort to examine the presence of any symbiosis-specific genes, expressed specifically during the ectomycorrhizal development, as this association presumably involves specific expression of both fungal and plant genes. Analysis of the soluble proteins from uninfected roots, free-living mycelia, and ectomycorrhizas by SDS–PAGE showed that the majority of the polypeptides observed in mycorrhizas were already synthesized in either uninfected root or mycelia. As in *Eucalyptus–Pisolithus* mycorrhiza, the development of *E. globulus–P. involutus* ectomycorrhizas was accompanied by the appearance or disappearance of some polypeptides, representing a minor fraction of the total soluble proteins in the ectomycorrhizas. It was, however, not possible to ascertain at that stage whether these ectomycorhiza-specific polypeptides were coded by fungal or plant genome. Here also, the name ectomycorrhizin was suggested for the class of ectomycorrhiza-specific polypeptides that were present in ectomycorrhizas but were undetectable in uninfected roots or free-living mycelia (Hilbert, Gaudin, Martin, *et al.* 1987).

VA-mycorrhiza-specific polypeptides

In studies conducted at the Botanisches Institut der Universitat Basel, Hebelstrasse 1, Basel, Switzerland, wild-type plants of the soybean cv. Bragg and two mutants derived from Bragg (nod 49 and nod 139), unable to form nodules with *Bradyrhizobium japonicum*, were compared for their reaction with *G. mosseae*. Their roots entered equally well into VA mycorrhiza symbiosis. Polyadenylated RNA was isolated from nodule-free mycorrhizal and non-mycorrhizal roots and translated in vitro. Antisera reacting with soluble or membrane-bound nodulins did not immunoprecipitate any of the translation products from non-mycorrhizal roots but they reacted with specific translation products from mycorrhizal roots of both wild-type and mutant plants. Two polypeptides (molecular weights 135–140 kDa and 18 kDa) were immunoprecipitated with the antiserum against soluble nodulins and three (21–28 kDa) with that against membrane-bound nodulins. These results indicate that symbiosis-specific polypeptides, possibly identical with nodulins, are induced in the mycorrhiza and can be termed 'mycorrhizins', as mentioned before (Wyss, Mellor, and Wiemken 1990).

In studies conducted at the University of Angers, Ufr Science, Interact Plantes Microorganismes, Lavoisier, Angers, France, Ri T-DNA (root-inducing transferred-DNA)-transformed roots of tomato (*Lycopersicon esculentum*) were in vitro inoculated with surface-sterilized VAM leek root pieces. About one week after inoculation, the infection of the transformed root culture by the fungal endophyte was confirmed by photonic microscopy. Total proteins were extracted from the mycorrhizal roots and analysed by 2D PAGE. Control gels were run with proteins extracted from non-inoculated roots mixed with purified intraradical vesicles and extraradical hyphae. Comparison of the resulting patterns revealed the presence of two polypeptides with estimated apparent masses of 24 kDa and 39 kDa that were detected only in infected roots. Polypeptides with similar migration parameters were not detected in roots challenged with spore extracts, suggesting that the accumulation of the polypeptides was directly linked to root colonization by the fungus rather than to induction by fungus-derived elicitors (Simoneau, Louisylouis, Plenchette, *et al.* 1994).

Genes controlling mycorrhiza-specific polypeptides

Ectomycorrhiza, a specialized root organ, is the result of a complex interaction leading to the finely tuned symbiosis between a plant and a compatible ectomycorrhizal fungus. Ultrastructural observations combined with cytochemical and biochemical studies reveal that structural and metabolic changes in the symbiont cells lead to the final phenotype of the active ectomycorrhiza. Recent genetic data indicates that the continued vegetative growth of the ectomycorrhizal hyphae and the root tissues and their ability to switch to symbiotic organ formation are basically controlled by developmentally critical genes (Martin, Hilbert, Henrion, *et al.* 1990).

Studies were conducted at the Laboratoire de Microbiologie Forestiere, Centre de Recherches Forestieres de Nancy, Institut National de la Recherche Agronomique, Champenoux, France to clone genes from the *E. globules–P. tinctorius* symbiosis, which control ectomycorrhiza formation. Isolation and characterization of these genes will provide molecular markers for the analysis of mycorrhiza development, and allow the basis of their tissue-specific regulation to

be investigated. As prerequisites to cloning these genes, changes in gene expression were characterized at the protein level, and symbiosis-specific polypeptides were identified. The most salient aspects during the differentiation in eucalyptus ectomycorrhizas are that (i) transition from free-living partners to symbionts is accompanied by a large decrease in polypeptide number and concentration and (ii) ectomycorrhiza formation coincides with a change in the expression of a few mRNAs that produce abundant symbiosis-specific polypeptides, the ectomycorrhizins. The expression pattern of early ectomycorrhizin genes during mycorrhiza development suggests that the products of these genes are involved in mycorrhiza morphogenesis since their appearance accompanies the symbiosis formation. Differential polyadenylation or differential rates of turnover of particular mRNAs cannot be distinguished from differential transcription of specific DNA sequences as yet. It will be of interest to determine to what extent changes in morphogenetic complexity and polypeptide biosynthesis in the two symbionts are associated with changes in gene transcription (Martin, Hilbert, Henrion, et al. 1990).

Further biochemical investigations conducted at the institute on *Eucalyptus–Pisolithus* symbiosis revealed drastic alterations experienced by fungal cell wall polypeptides during the early stages of the ectomycorrhizal interaction. Differential screening of a mycorrhiza cDNA library and large-scale cDNA sequencing allowed the characterization of several symbiosis-regulated genes in aerial hyphae and during mantle formation. The cell wall of the fungal partner presented a novel protein composition, characteristic of the symbiotic status. A convergence of biochemical and molecular results allowed formulation of molecular models for the key role of the cell wall compartment in the early developmental stages of ectomycorrhizal formation (Tagu and Martin 1996).

In studies conducted at the Laboratoire des Interactions Plantes-Microorganismes, Universite d'Angers, 2 Boulevard Lavoisier, Angers, France, micropropagated plantlets of birch (*B. pendula*) were inoculated with seven different isolates of the mycorrhizal fungus, *P. involutus*. Based on the level of fungal ergosterol measured in roots at the end of the mycorrhizal formation stage, a strain PO was chosen as the reference strain. Electrophoretic analysis of in vivo labelled proteins extracted from mycorrhizal roots 96 hours post-inoculation with this strain, non-inoculated roots, and free-living mycelium revealed that specific polypeptides were synthesized during ectomycorrhiza formation. To examine hypothetical similarity between some of these polypeptides and defence proteins, parts of corresponding putative genes of birch were isolated. Partial sequencing of one clone has shown that it contained a portion of the gene for phenylalanine ammonialyase (Simoneau, Juge, Dupuis, *et al*. 1994).

In studies conducted at the Samuel Roberts Noble Federation Incorporated, Division of Plant Biology, Ardmore, OK, USA, three cDNA clones representing genes, expression of which is induced during the AM symbiosis formed between *Medicago truncatula* and an AM fungus, *Glomus versiforme*, were identified. The three clones represent *M. truncatula* genes and encoded novel proteins: a xyloglucan-endotransglycosylase-related protein, a putative AGP (arabinogalactan) protein, and a putative homologue of the mammalian p110 subunit of eIF3 (initiation factor 3). These genes showed little or no expression in *M. truncatula* roots prior to the formation of the symbiosis and were significantly induced following colonization by *G. versiforme*. The genes were not induced in roots in response to increases in phosphate. This suggested that induction of expression during the symbiosis was due to the interaction with the fungus and was not a secondary effect of improved phosphate nutrition. In situ hybridization revealed that the putative AGP is expressed specifically in cortical cells containing arbuscules. The identification of two mycorrhiza-induced genes encoding proteins estimated to be involved in cell wall structure was consistent with previous electron microscopy data that indicated major alterations in the extracellular matrix of the cortical cells following colonization by mycorrhizal fungi (vanBuuren, MaldonadoMendoza, Trieu, *et al*. 1999).

Symbiosis-specific changes in mycorrhizal roots

In studies conducted at the Murdoch University, School of Biological Sciences, Perth, Australia, protein biosynthesis in *Pisolithus–E. grandis* ectomycorrhiza was related to the stage of ectomycorrhizal development using 2D PAGE of proteins labelled with in vivo incorporation of ^{35}S-radiolabelled amino acids. Nineteen-day-old seedlings were radiolabelled and the primary root was divided into 1-cm segments. With increasing distance from the tip of the primary root, the lateral roots developed as follows: segment 1, no lateral tips; segment 2, three lateral tips, 1–4 days old; segment 3, five lateral tips, 3–8 days old; and segment 4, five lateral tips, 7–12 days old. Six-day-old ectomycorrhizas were fully formed with a mantle and Hartig net. During ectomycorrhizal development, there was a decrease in all plant

proteins and differential accumulation of fungal proteins. The apical segment of the primary root had a biosynthesis profile very similar to that of the non-inoculated roots. By contrast, the other segments of the primary root, with attached lateral roots, had biosynthesis profiles that were similar to those of the free-living hyphae. Thus, the plant biosynthesis was shown to be predominantly associated with the primary root meristem. The domination of the fungal partner in the protein biosynthesis of developing ectomycorrhiza was probably a consequence of stimulated fungal growth and the corresponding decrease in plant meristematic activity. Ectomycorrhizal development was associated with a differential accumulation of fungal polypeptides and the appearance of a group of symbiosis-related acid fungal polypeptides with molecular weights between 27 kDa and 37 kDa. As the polypeptides were present in a similar magnitude throughout ectomycorrhizal development (lateral tips 1–12 days old), it was suggested that they functioned as structural proteins associated with mantle formation (Burgess and Dell 1996).

Studies conducted at the University of Henri Poincare, Faculty of Science, Laboratory Biology Forestiere, INRA, Vandoeuvre, Nancy, France, on seedlings of *B. pendula* grown in the presence of *P. involutus* to study metabolic changes during mycorrhiza formation showed that glutamine, aspartate, and asparagine pools were always lower in infected roots than in non-infected roots, especially during Hartig net initiation and formation. Glutamate concentration was similar in both tissues. Citrate and malate were two major organic acids detected, and their concentrations were equal in infected and non-infected roots. Aspartate aminotransferase, glutamine synthetase, NAD (nicotinamide adenine dinucleotide)-dependent malate dehydrogenase, and glucose-6-phosphate dehydrogenase activities were higher in infected roots than in non-infected roots. For all enzymes revealed on PAGE, both root and fungal isoforms were present in infected roots. Quantitative changes in enzyme capacities and metabolite pools indicated that mycorrhiza formation caused a re-arrangement of the main metabolic pathways during the very early stages following contact, which might be related to the structural changes (Blaudez, Chalot, Dizengremel, *et al*.1998).

Studies conducted at the University of Helsinki, Department of Bioscience, Division of Plant Physiology, Viikinkaari, Helsinki, Finland, on analysis of total and radiolabelled proteins from *Pinus sylvestris* short roots, ectomycorrhiza, and the ectomycorrhizal fungus, *Suillus bovinus*, by 2D gel electrophoresis showed that the specialized growth pattern of short roots was associated with production of five short-root-specific proteins. Several proteins of the main and lateral roots were repressed in short roots. At the morphologically different stages of the ectomycorrhiza, only few changes appeared in the amounts of the host and the symbiont proteins. Only one ectomycorrhiza-specific protein could be distinguished (Tarkka, Niini, and Raudaskoski 1998).

In studies conducted at the Dipartimento di Biologia Vegetale, Universita degli Studidi Torino, Turin, Italy, the presence of HRGP (hydroxyproline-rich glycoproteins) at the interface was investigated with a polyclonal antibody obtained against melon callus HRGP2b. Using a combination of cytochemical methods, antigens were detected in peas, in the presence and absence of *G. versiforme*, a VAM fungus. For comparison, experiments were performed in parallel, using leeks as a monocot host. Antigens were localized over the pea cell walls in root tissues. At the ultrastructural level, gold granules were mostly present in the periplasmic space. In mycorrhizal plants, the most substantial deposition occurred at the surface between the fungal wall and the host membrane. In leeks, labelling was limited to cell walls and did not change in the presence of *G. versiforme*. Dot blot experiments revealed that HRGP2b antigens could be localized over the cell wall of both dicot and monocot hosts, although they mostly occur in the contact zone in infected samples, and their presence in association with localized glucans and pectins meant that the contact zone could be regarded as an apoplastic space, presenting a structural response to the symbiotic mycorrhizal status (Bonfante-Fasolo, Tamagnone, Peretto, *et al*. 1991).

Studies conducted at the Soil Microbial Systems Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, USA, on material on the surface of hyphal walls of VAM fungi during active colonization of plant roots detected by a monoclonal antibody showed that pot-cultured isolates of Glomus, Acaulospora, Gigaspora, Scutellospora, and Entrophospora had IM (immunofluorescent material) on younger, thinner, intact hyphae, but IM was scant to absent on thicker, melanized or lysing hyphae. Colonization of maize, sudangrass (*Sorghum sudanense*) or red clover (*Trifolium pratense*) was examined during five months of plant growth by removing cores and performing an indirect immunoassay on roots with attached hyphae. Fresh spores of some *Glomus* spp. had IM on the outer layer of the spore wall. Abundant IM was seen on root hairs of plants colonized by some isolates, and some IM was detected on root surfaces of all plants examined even during early colonization. After

cultures were dried, hyphae, roots, and spores had little to no IM. Uninoculated control roots had very rare, small patches of IM. An immunoreactive protein was extracted from hyphae of Gigaspora and Glomus isolates by using 20 mM citrate (pH 7.0) at 121 ºC for 90 minutes. Gel electrophoresis profiles indicated that all isolates tested had the same banding patterns. Lectin binding of extracted protein was suggestive of a glycoprotein (Wright, Franke-Snyder, Morton, *et al*. 1996).

Role of tubulin and actin in ectomycorrhiza development

In studies conducted at the Department of Botany, University of Helsinki, Unioninkatu, Helsinki, Finland, on ectomycorrhizal associations between pine (*P. sylvestris* and *Pinus contorta*) seedlings and *S. bovinus* or *P. involutus*, immunoblotting of polypeptides separated from crude tissue extracts by electrophoresis revealed an abundance of tubulin and actin in ectomycorrhiza and lower amounts in the fungal strands surrounding the ectomycorrhizal roots. The alpha-tubulins from fungal hyphae and plant cells were clearly distinguishable in ectomycorrhiza but such discrimination was not possible for beta-tubulin or actin due to the similar mobility of proteins originating from the conifer and fungal tissues. Microtubules were detected in both the conifer cells and in fungal hyphae in young ectomycorrhizal short roots (fixed while still attached to the seedlings) using indirect immunofluorescence microscopy with tubulin antibodies. Cytoplasmic and spindle microtubules were visualized in meristem cells and in differentiating vascular tissue in the host plant but not in the cortical cells. In symbiotic hyphae, the microtubule tracks and spindles of dividing nuclei were clearly distinguished in the mantle hyphae in the tip region of the short roots. Microtubule tracks changed to a less clear, reticulate structure in the Hartig net hyphae. Actin was visualized as long filaments in vascular tissue cells and as small microfilament bundles in mantle hyphae. Short microtubules and actin dots were detected in cytoplasm-containing hyphae on the strand surface (Timonen, Finlay, Soderstrom, *et al*. 1993).

Further studies in the Department of Biosciences in the above-mentioned University on the role of tubulin and actin in the development of Scots pine (*P. sylvestris*) roots and in the formation of the ectomycorrhiza with *S. bovinus* showed that in the short roots, the alpha-tubulin pattern, as determined by immunoblotting of 2D gels with anti-tubulins and anti-actin antibodies, was different from that in the other root types due to the more acidic pi of the two alpha-tubulins. During the formation of the ectomycorrhiza, two new alpha-tubulins were detected in the acidic alpha-tubulin cluster. No such variation occurred in the plant with beta-tubulin patterns. The fungal tubulins dominated in the ectomycorrhiza, but no changes in tubulin polypeptide patterns were observed. Contrary to the tubulins, plant actin dominated in the mycorrhiza. The specific alpha-tubulin patterns of uninfected and infected short roots indicated that alpha-tubulin was involved in the morphogenesis of *P. sylvestris* short roots. The high level of plant actin at early stage of the mycorrhiza formation suggested a significant role of this protein in the interaction between plant cells and fungal hyphae (Niini, Tarkka, and Raudaskoski 1996).

In studies conducted at the Plant Physiology Lab, Rockville, Md, USA, a lectin (LDetL) was isolated from carpophores of the mushroom, *Lactarius deterrimus*, a specific symbiont of the spruce, by a combination of affinity, hydroxylapatite, and gel-filtration chromatography. The molecular mass, as determined by gel filtration, was about 37 000 Daltons, and its structure was dimeric, with two identical subunits assembled by noncovalent bonds. It appeared homogeneous on high-performance liquid chromatography gel filtration, but isoelectric focusing revealed microheterogeneity, with a main band in the pH zone near 6.5. Amino acid analysis showed that LDetL contained a large proportion of glycine, especially methionine. Hapten inhibition assay indicated that LDetL was most specific for beta-D-galactosyl (1 leads to 3)-D-N-acetyl galactosamine residues. The lectin was formed in the in vitro cultivated mycelium, and anti-lectin antibodies revealed the presence of lectin in the cell wall by indirect immunofluorescence. Receptor sites for LDetL were found on the roots, especially on the root hairs, of axenically grown spruce seedlings. The lectin LDetL previously isolated by the authors from the taxonomically related mushroom *Lactarius deliciosus*, a symbiont of the pine, did not bind to the spruce radicle. This suggested a role of the fungal lectin in recognition and specificity during the early stages of mycorrhizae formation (Giollant, Guillot, Damez, *et al*. 1995).

In studies conducted at the University of Helsinki, Department of Botany, Unioninkatu, Helsinki, Finland, on *G. mosseae*, microtubules and microfilaments were visualized by indirect immunofluorescence microscopy in hyphae—quick-

frozen, freeze-substituted, and treated with cell-wall-degrading enzymes. Microtubules were distinguished in both the cortical and the central parts of hyphae, while microfilaments were revealed only in the cortical part. In immunoblotting, tubulin and actin were detected in an extract from hyphae elicited by host plants (Astrom, Giovannetti, and Raudaskoski 1994).

References

Astrom H, Giovannetti M, and Raudaskoski M. 1994
Cytoskeletal components in the arbuscular mycorrhizal fungus *Glomus mosseae*
Molecular Plant–Microbe Interactions **7**(2): 309–312

Blaudez D, Chalot M, Dizengremel P, Botton B.1998
Structure and function of the ectomycorrhizal association between *Paxillus involutus* and *Betula pendula* – II. Metabolic changes during mycorrhiza formation
New Phytologist **138**(3): 543–552

Bonfante-Fasolo P, Tamagnone L, Peretto R, Esquerre-Tugaye M T, Mazau D, Mosiniak M, Vian B. 1991
Immunocytochemical location of hydroxyproline rich glycoproteins at the interface between a mycorrhizal fungus and its host plant
Protoplasma **165**(1–3): 127–138

Burgess T and Dell B. 1996
Changes in protein biosynthesis during the differentiation of *Pisolithus–Eucalyptus grandis* ectomycorrhiza
Canadian Journal of Botany – Revue Canadienne de Botanique **74**(4): 553–560

Burgess T, Laurent P, Dell B, Malajczuk N, Martin F. 1995
Effect of fungal-isolate aggressivity on the biosynthesis of symbiosis-related polypeptides in differentiating eucalypt ectomycorrhizas
Planta **195**(3): 408–417

Dassi B, Samra A, Dumas-Gaudot E, Gianinazzi S. 1999
Different polypeptide profiles from tomato roots following interactions with arbuscular mycorrhizal (*Glomus mosseae*) or pathogenic (*Phytophthora parasitica*) fungi
Symbiosis **26**(1): 65–77

Dumas E, Goamomazzo-Pearson V, and Gianinazzi S. 1988
Changes in host-coded proteins during endomycorrhiza formation
[Second European Symposium on Mycorrhiza, Prague, Czechoslovakia, 14–20 August 1988]

Dumas-Gaudot E, Guillaume P, Tahiri-Alaoui A, Gianinazzi-Pearson V, Gianinazzi S. 1994
Changes in polypeptide patterns in tobacco roots colonized by two *Glomus* species
Mycorrhiza **4**(5): 215–221

Garcia-Garrido J M, Toro N, and Ocampo J A. 1993
Presence of specific polypeptides in onion roots colonized by *Glomus mosseae*
Mycorrhiza **2**(4): 175–177

Giollant M, Guillot J, Damez M, Dusser M, Didier P, Didier E. 1995
Characterization of a lectin from *Lactarius deterrimus*. Research on the possible involvement of the fungal lectin in recognition between mushroom and spruce during the early stages of mycorrhizae formation
Plant Physiology **101**(2): 513–522

Henrion B and Martin F. 1990
Changes in membrane polypeptides and glycoproteins during eucalypt mycorrhiza development
Wyoming: University of Wyoming. p.198
[Eighth North American Conference on Mycorrhiza Innovation and Hierarchical Integration, Jackson, Wyoming, USA, 5–8 September 1990, organized by the University of Wyoming and San Diego States University]

Hilbert J L and Martin F.1988a
Regulation of gene expression in ectomycorrhizas. I. Protein changes and the presence of ectomycorrhiza-specific polypeptides in the *Pisolithus–Eucalyptus* symbiosis
New Phytologist **110**(3): 339–346

Hilbert J L and Martin F. 1998b
Changes in polypeptide profiles during the establishment of ectomycorrhizal symbiosis
Cryptogamie Mycologie **9**(3): 221–231

Hilbert J L and Martin F. 1988c
Protein changes and the presence of ectomycorrhiza-related proteins in the *Pisolithus–Eucalyptus* symbiosis
[Second European Symposium on Mycorrhizae, Prague, Czechoslovakia, 14–20 August 1988]

Hilbert J L and Martin F. 1990
Protein changes and the presence of ectomycorrhiza-specific polypeptides in the *Pisolithus–Eucalyptus* symbiosis
Agriculture, Ecosystems and Environment **28**(1/4): 181–184

Hilbert J L, Costa G, and Martin F. 1991
Ectomycorrhiza in synthesis and polypeptide changes during the early stage of eucalypt mycorrhiza development
Plant Physiology **97**(3): 977–984

Hilbert J L, Gaudin V, Martin F, Lapeyrie F. 1987
Identification of 'ectomycorrhiza-specific' proteins (ectomycorrhizins) involved in the development of *Paxillus–Eucalyptus* symbiosis
[Seventh North American Conference on Mycorrhiza, Gainesville, Florida, 3–8 May 1987]

Martin F M, Hilbert J L, Henrion B, Costa G. 1990
Biochemical and molecular changes during ectomycorrhiza development
Wyoming: University of Wyoming. p. 200
[Eighth North American Conference on Mycorrhiza Innovation and Hierarchical Integration, Jackson, Wyoming, USA, 5–8 September 1990, organized by the University of Wyoming and San Diego States University]

Niini S S, Tarkka M T, and Raudaskoski M.1996
Tubulin and actin protein patterns in Scots pine (*Pinus sylvestris*) roots and developing ectomycorrhiza with *Suillus bovinus*
Physiologia Plantarum **9**6(2): 186–192

Samra A, Dumas-Gaudpt E, and Gianinazzi S. 1997
Detection of symbiosis-related polypeptides during the early stages of the establishment of arbuscular mycorrhiza between *Glomus mosseae* and *Pisum sativum* roots
New Phytologist **135**(4): 711–722

Simoneau P, Louisylouis N, Plenchette C, Strullu D G. 1994
Accumulation of new polypeptides in Ri T-DNA-transformed roots of tomato (*Lycopersicon esculentum*) during the development of vesicular-arbuscular mycorrhizae
Applied and Environmental Microbiology **60**(6): 1810–1813

Simoneau P, Viemont J D, Moreau J C, Strullu D G. 1993
Symbiosis-related polypeptides associated with the early stages of ectomycorrhiza organogenesis in birch (*Betula pendula* Roth)
New Phytologist **124**(3): 495–504

Simoneau P, Juge C, Dupuis J Y, Viemont J D, Moreau J C, Strullu D G. 1994
Protein biosynthesis changes during mycorrhiza formation in roots of micropropagated birch
Acta Botanica Gallica **141**(4): 429–435

Tagu D and Martin F.1996
Molecular analysis of cell wall proteins expressed during the early steps of ectomycorrhiza development
New Phytologist **133**(1): 73–85

Tarkka M, Niini S S, and Raudaskoski M. 1998
Developmentally regulated proteins during differentiation of root system and ectomycorrhiza in Scots pine (*Pinus sylvestris*) with *Suillus bovinus*
Physiologia Plantarum **104**(3): 449–455

Timonen S, Finlay R D, Soderstrom B, Raudaskoski M. 1993
Identification of cytoskeletal components in pine ectomycorrhizas
New Phytologist **124**(1): 83–92

vanBuuren M L, MaldonadoMendoza I E, Trieu A T, Blaylock L A, Harrison M J. 1999
Novel genes induced during an arbuscular mycorrhizal (AM); symbiosis formed between *Medicago truncatula* and *Glomus versiforme*
Molecular Plant–Microbe Interactions **12**(3): 171–181

Wright S F, Franke-Snyder M, Morton J B, Upadhyaya A. 1996
Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots
Plant and Soil **181**(2): 193–203

Wyss P, Mellor R B, and Wiemken A. 1990
Vesicular-arbuscular mycorrhizas of wild-type soybean and non-nodulating mutants with *Glomus mosseae* contain symbiosis-specific polypeptides (mycorrhizins), immunologically cross-reactive with nodulins
Plant **182**(1): 22–26

Research finding papers

Variation of arbuscular mycorrhizal status of plants with the moisture gradient in slopes of the River Bhagirathi in summer and monsoon

Somdatta Ghosh*, Ruma Biswas, Haimanti Bhattacharya, N K Verma
Department of Botany and Forestry, Vidyasagar University, Midnapur – 721102, West Bengal, India

Introduction

Plant community is determined by AM (arbuscular mycorrhizal) association. AM fungi are essential for any community (Francis and Read 1994). It is reported that wetland plants have poor colonization (Raghupati and Mahadevan 1993). AM colonization and spore density also differ with season, edaphic conditions, and plant species (Ghosh and Verma 2002, 2004). The transition zone between the aquatic and the terrestrial region along a river bank shelters moisture-loving plants. In monsoon, this zone is immersed in water. This study was conducted to measure the changes of colonization pattern and spore density in three zones with different moisture levels, along the river bank of Bhagirathi in Murshidabad, West Bengal, in two seasons—summer and monsoon. Effect of moisture on AM association in field plants with seasonal variation is not well reported (Brundrett and Abbott 1994).

Materials and methods

This study was carried out with a line transect drawn along the slope of the river from water level to 10 m upwards. This length was divided into three zones – aquatic, middle, and upper zones – each with 3.3 m length. Sampling was done during low tides. Soil samples were collected in three replicates for each zone. Transect was drawn three times at 100-m intervals. Root samples were collected in polythene bags. Soil samples were tested for pH, moisture content, EC (electrical conductivity), organic carbon, P (phosphorus), and K (potassium) content (Jackson 1973). AM spores were isolated and counted (Gerdemann and Nicolson 1963).

Root samples were washed thoroughly. Fine roots were cut into 1-cm pieces, treated with 10% potassium hydroxide solution, and stained with 0.5% tryphan blue solution (Phillips and Hayman 1970). Root pieces were observed for colonization (×100). Colonization percentage was measured by the following formula.

- Number of root pieces infected/Number of root pieces observed ×100
- Fifty root pieces were observed for each plant species. Statistical analyses were done with the help of 'statistica'.

Results and discussion

Soil P and K content, and organic carbon were observed to decrease gradually from the upper to the lower region (Table 1). After rain, EC, P, K, and moisture content increased in all the three zones while organic carbon decreased. pH was reported to be neutral in both the seasons. Spore

Table 1 Physical and chemical properties of soil in three zones of river slope of the Bhagirathi in summer and monsoon

	Summer						*Monsoon*					
Zone	*pH*	*EC* (dS/m)	*Organic carbon* (%)	P_2O_5 (kg/ha)	K_2O (kg/ha)	*Moisture content* (%)	*pH*	*EC* (dS/m)	*Organic carbon* (%)	P_2O_5 (kg/ha)	K_2O (kg/ha)	*Moisture content* (%)
Aquatic zone	7.3	0.5	0.405	100	190	30	7	0.97	0.24	188	450	30
Middle zone	7.3	0.7	0.495	100	297	20	7	0.80	0.26	180	450	23
Upper zone	7.3	0.57	0.66	110	450	17	7	0.87	0.57	176	450	21

P_2O_5 – phosphorus pentoxide; K_2O – potassium oxide; EC – electrical conductivity

* E-mail: somdattaghosh@yahoo.co.in

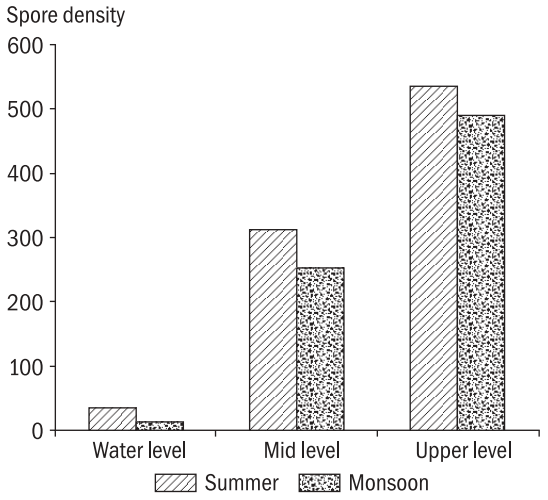
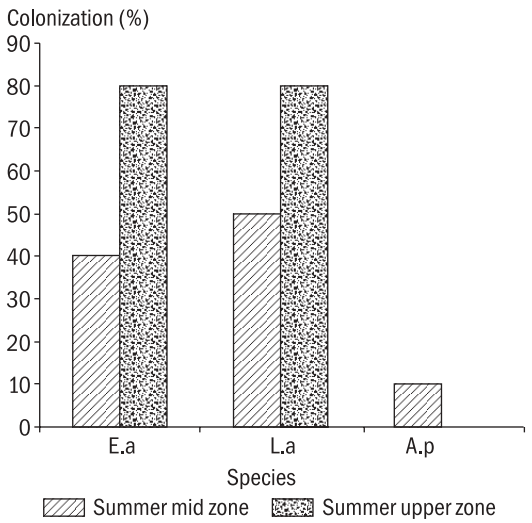


Figure 1 Spore density in three zones in river bank in summer and monsoon

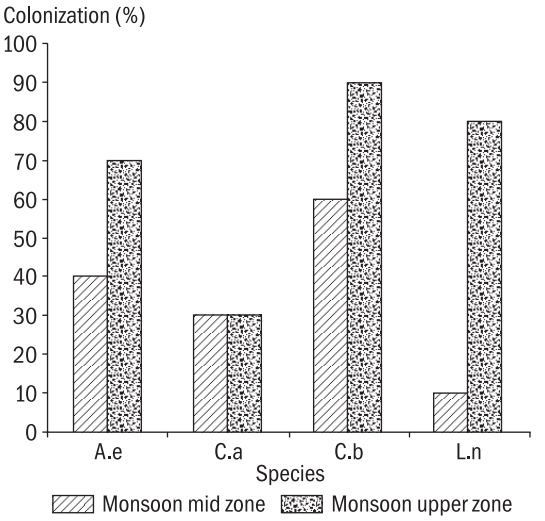
density was observed to decrease from the upper to the lower zones in both the seasons after rain (Figure 1). Spore density showed significant negative correlation (–0.96; $P<0.05$; $N = 60$) with moisture.

In summer, in aquatic zone, three non-mycorrhizal species – *Polygonum hydropiper*, *Panicum repense*, and *Ipomea aquatica* – were observed, though AM spores were present in soil, probably washed down from the upper zones. Colonization percentage was reported to be higher in the plants of the upper



E.a – *Eclipta alba;* L.a – *Lippia alba;* A.p – *Alternanthera phylaxoides*

Figure 2 Colonization percentage in the upper and middle zones in summer



A.e – *Alocasia esculaenta*; C.a – *Centella asiatica*; C.b – *Croton bonplandianum*; L.n – *Lippia nodiflora*

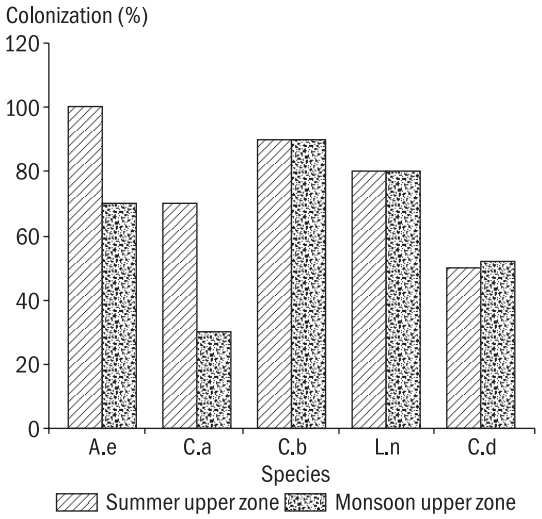
Figure 3 Colonization percentage in the upper and middle zones in monsoon

zone compared to those of the middle zone (Figure 2). This is obviously due to moisture stress as P content in both the zones was same. Moisture stress increases AM dependency in host plants (Hetrick, Kitt, and Wilson 1987). Only *Alternanthera philoxides*, a wetland plant generally accepted as non-mycorrhizal, showed mycotrophy in moist soil.

High moisture content also reduced colonization in the middle zone compared to the upper zone in monsoon (Figure 3). The only exception was observed in *Centella asiatica* wherein colonization percentage was not affected by moisture. After rain, as water level rises, some plants of the middle zone in summer get immersed and are included in the aquatic zone. This conversion compels the semi-aquatic plants to grow in high moisture content (30%) and colonization percentage decreased up to 40% compared to the middle zone.

Other than moisture content, EC and P content also increased in the upper zone after rain. High P in soil is reported to reduce AM development (Hetrick, Wilson, and Todd 1996). In this case, changed soil conditions had no effect on the colonization of terrestrial plants and little effect on semi-aquatic species – *C. asiatica* and *Alocasia esculenta* – that reported a decrease in colonization (Figure 4). The results indicate that colonization in wetland plants is more sensitive to moisture. These plants are more dependent on AM in moisture and nutrient stress.

Moisture gradient showed a negative correlation with colonization percentage in summer (–0.86; $P<0.05$) and monsoon (–0.72; $P<0.05$). Increased moisture, more than optimum level, may decrease



A.e - *Alocasia esculaenta*; C.a - *Centella asiatica*; C.b - *Croton bonplandianum*; L.n - *Lippia nodiflora*; C.d - *Cynodon dactylon*

Figure 4 Colonization percentage in the upper zone in summer and monsoon

colonization (Readhead 1975; Sieverding 1984). Sporulation and colonization showed higher correlation in summer (0.91) than monsoon (0.72). Decreased colonization after rain, however, was contrary to the findings of acidic soil (Ghosh and Verma 2004).

References

Brundrett M C and Abbott L K. 1994
Mycorrhizal fungus production in the Jarrah forest. I. Seasonal study of inoculum level
New Phytologist **127**: 539–546

Francis R and Read D J. 1994
The contribution of mycorrhizal fungi to the determination of plant community structure
Plant soil **159**: 11–25

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

Ghosh S and Verma N K. 2002
A preliminary report on VA mycorrhizal status of some common weeds, trees and aquatic plants of south west Bengal
Science culture **68**(5–6): 155–157

Ghosh S and Verma N K. 2004
Seasonal variation of arbuscular mycorrhizal status in rhizosphere of six plantation species grown in lateritic soil of south west Bengal
Mycopath Research **42**(2): 229–232

Hetrick B A D, Kitt D G, and Wilson G T. 1987
Effect of drought stress on growth response in corn, Sudan grass and big blue stem to *Glomus etunicatum*
New Phytologist **105**: 403–410

Hetrick B A D, Wilson G W T, and Todd T C. 1996
Mycorrhizal response in wheat cultivers, relationship to phosphorus
Journal of Botany **1**: 19–25

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

Phillips J M and Hayman D S. 1970
Improved procedure for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection
Transaction of British Mycological Society **55**: 158–161

Raghupati S and Mahadevan A. 1993
Distribution of vesicular arbuscular mycorrhiza in the plants and rhizosphere soils of tropical plains in Tamil Nadu, India
Mycorrhiza **3**: 123–136

Readhead J E. 1975.
Endomycorrhizas
New York: Academic press. 447 pp.

Sieverding E.1984
Influence of soil water regimes on VA mycorrhiza. III. Comparison of three mycorrhizal fungi and their influence on transpiration
Z. Acker pflanzenbau. **153**: 52–61

Pattern of mycorrhizal infections in the roots of *Aerides maculosum* Lindl. and *Calanthe triplicata* (Willem.) Ames

S Kaliamoorthy
Botanical Survey of India, Southern Circle, Coimbatore – 641 003, Tamil Nadu, India

Introduction

The term mycorrhiza, as explained by many workers, describes the association between the roots of a terrestrial plant and one or more species of fungi (Weber and Webster 2001). With the exception of hydrophytes and a few families of land plants, all other plants and inhabitants of dry soil are reported to possess mycorrhiza (Burgeff 1959). The possession of mycorrhiza is very essential for plants growing in nutrient-poor soil as the fungal mycelium helps in the translocation of mineral nutrients from the soil, making them available to the plants. Orchids are very unique in the possession of mycorrhiza because in all natural situations, they depend on mycorrhizal fungi either throughout their life cycle or at least until the initial establishment of seedlings (Clements 1988; Smith and Read 1997; Weber and Webster 2001). In view of this, orchids have been described as mycotrophs (fungus-feeders) by Zettler (1997). The phenomena of mycotropism and mycorrhiza are described by many workers as the part of terrestrial orchid species, particularly of temperate and sub-tropical forms (Arditti 1966; Dressler 1981; Hadley 1984; Rasmussen 1995). In epiphytic forms of tropical areas, the phenomena of mycotropism and mycorrhiza are considered to be less important because of their above-ground habit that ensures greater access to sunlight for photosynthesis (Rasmussen 1995). Although voluminous works have already been published on the natural infection patterns in orchids, work hitherto on tropical species is very scarce (Hadley 1984; Benzing 1982; Hadley and Williamson 1972; Burgeff 1959). The present work is, therefore, undertaken with an objective of understanding the pattern of natural infection in epiphytic and terrestrial orchid species growing in the existing tropical climate of peninsular India.

Materials and methods

The plant materials used for the present study like *Aerides maculosum* Lindl., an epiphytic orchid, and *Calanthe triplicata* (Willmet) Ames, a terrestrial orchid, were collected from the germplasm maintained at the National Orchidarium and Experimental Garden, Botanical Survey of India, Yercaud, Salem district, Tamil Nadu, India. The young and old roots of these orchid species with mycorrhizal infections were used. In *C. triplicata*, the roots present underneath the soil surface were harvested. In the case of *A. maculosum*, the roots that were attached or clinged to the support were collected. The collected roots of both the species were washed thoroughly under running tap water for 10 minutes, to remove any soil particles or debris adhered to them, and were fixed separately in small pieces of about 2–3 cm in 5 ml formalin:5 ml acetic acid:90 ml 70% alcohol (FAA). The fixed roots were then used for mycorrhizal study. Observations were made with free hand transverse sections stained with toluidine blue O (Gahan 1984) (0.05% in acetate buffer, pH 4.4). The results were recorded for the percentage of colonization, as described by Hadley and Williamson (1972) and the state of pelotons, that is, number of undigested and digested pelotons. In addition, a preliminary account on the cytological changes of infected cells was made using the procedures, as outlined in Krishnamurthy (1999) (Table 1). Photomicrographs of the root sections were taken using Nikon E 400 (Japan) binocular microscope. For all the features recorded, 100 random measurements were made. Mean and standard deviation of the measurements in each parameter were assessed and presented.

Results and discussion

Root morphology

In *A. maculosum*, the root system was found to be well developed, greenish, and cylindrical in shape. However, the roots that were attached with the support were found to be flattened on one side. Groove was formed in old roots; might be due to the decay of epidermal cells. The infected portion appeared to be yellow in colour. In *C. triplicata*, there was a well-developed root system. The roots that were exposed above the ground level were found to be

Table 1 Cytochemical procedures to localize substances in the mycorrhiza infected roots

Substances	*Method employed*	*Result*	*Reference*
DNA	TBO (toluidine blue O) (0.05% TBO in acetate buffer)	DNA stains blue or blue-green	McCully (1966)
Lignin	TBO (toluidine blue O) (0.05% TBO in acetate buffer)	Lignin stain greenish blue	O'Brien, Feder, and McCully (1964)
Total proteins	CBB (coomassie brilliant blue) (0.02% CBB in Clarke's* solution)	Protein stains blue	Fisher (1968)
Lipids	NBS (Nile blue sulphate)	Phospholipidsstain blue	Gahan (1984)

*1 part glacial acetic acid: 3 parts absolute ethanol (Gahan 1984)

greenish. The infected zone of root system appeared to be slightly yellowish-orange in colour.

Mode of fungal entry and colonization

The transverse sections of infected roots of *A. maculosum*, which were in contact with the support, showed that the fungal entry could be through the distorted portion of root epidermal cells (Figure 1). The fungal hyphae traverse the velamen tissues of two to three cell layers before reaching the exodermal region. In the exodermis, the fungal hyphae were found to enter the cortical region through the passage cells. The cell walls of these passage cells were not lignified as in the other cells of the exodermis. This was confirmed cytochemically using toluidine blue O (O'Brien, Feder, and McCully 1964; Gahan 1984). Unlike in *A. maculosum*, the mode of entry in *C. triplicata* was observed to be through root hair (Figure 2). The fungal entry through the root hair has already been emphasized by Senthilkumar and Krishnamurthy (1998) in *Spathoglottis plicata* and by Weber and Webster (2001) in *Dactylorhiza maculata* ssp. *ericetorum*. However, the rest of the process that leads fungal hyphae to enter the cortical region was in the manner as observed in *A. maculosum*. Soon after its entry into the cortical region, the fungal colonization begins in the inner cortical cells.

The colonization of the fungus in the host root system was calculated as per the formulae given by

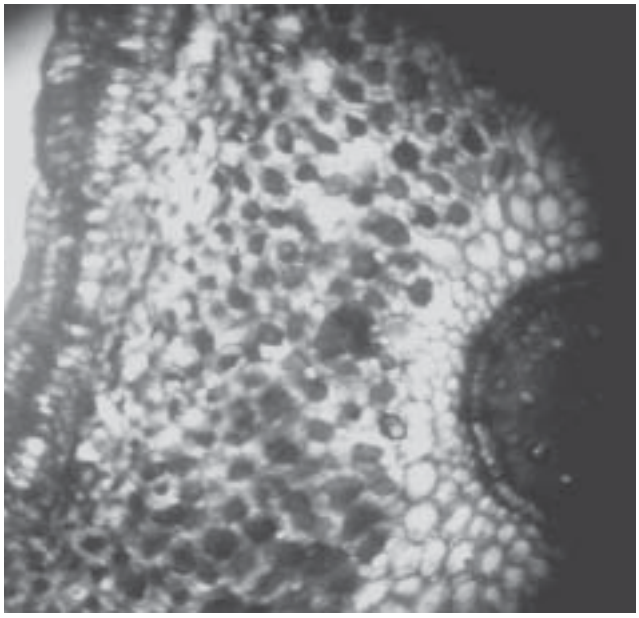


Figure 1 Transverse section of infected root of *Aerides maculosum* showing the distorted portion of epidermis (arrow), the possible entry point of fungus. Note the fungal colonization of the region clinging to the support

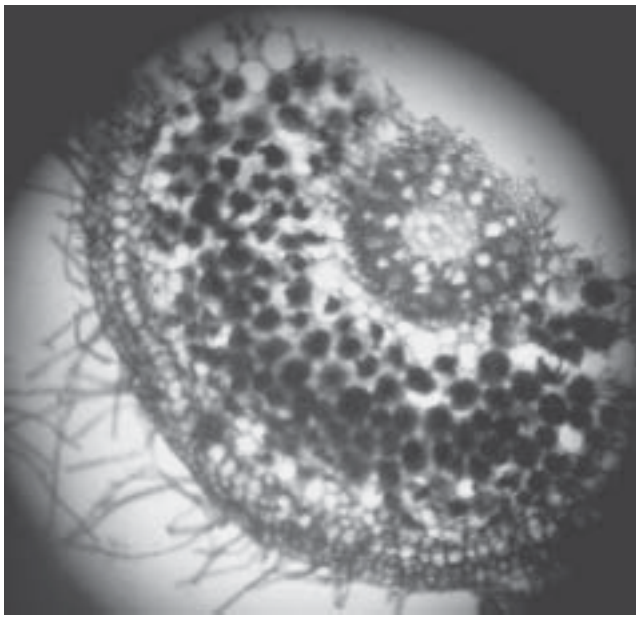


Figure 2 Transverse section of infected root of *Calanthe triplicata* showing the fungal colonization throughout the cortical region

Hadley and Williamson (1972). The results are presented in Table 2. The mean percentage of colonization was recorded from the infected zone of young and old roots. The results showed that in *A. maculosum*, the mean percentage was little more than 15% and 19%, respectively, in young and old roots (Figure 1). On the other hand, *C. triplicata* recorded a mean percentage little higher than 76% and 87%, respectively, in young and old roots (Figure 3). In his study on epiphytic Florida orchids, Benzing (1982) has reported that 10% or more of the entire cortex was involved with mycorrhizal infection. He further emphasized that the infection particularly lies with the part adjacent to the support. A similar result was noticed in the present study with *A. maculosum*. Although, in the present study, the older roots recorded marginally a higher percentage of colonization, this result may not support the view of Benzing (1982) that the older roots of epiphytic orchids carried the heaviest infection. Further, fungal colonization was observed right from the outermost cortex layer up to a maximum of six to seven layers of cells. The fungal colonization was found to be nearly localized. Although the latter statement may sound similar to the view of Hadley (1984), his opinion about the restriction of infection to the groups of two or three cells in the outer cortex may not be true in all the epiphytes. In *C. triplicata*, the colonization was almost noticed in the entire cortex region, leaving just one or two layers above the endodermis vacant; may be considered as storage layers (Burgeff 1959).

Cytological changes in the mycorrhizal infected cells

The fungal hyphae were observed as tightly interwoven coils called pelotons. Pelotons are considered to be the most distinctive characteristic of an orchid mycorrhiza (Currah and Zelmer 1992) and reflect the establishment of a successful, stable symbiosis (Zettler 1997). Apart from the usual

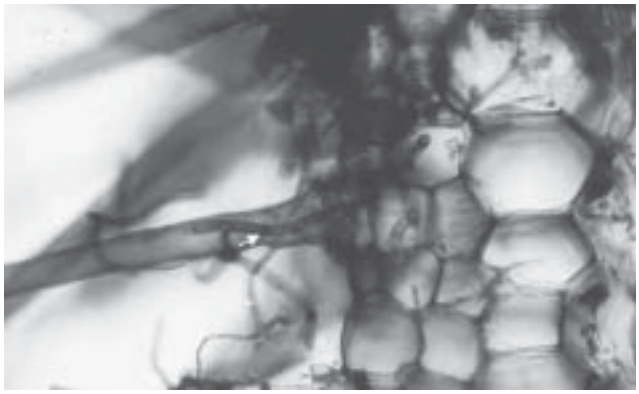


Figure 3 Transverse section of infected root of *Calanthe triplicata* showing the fungal hyphae entering through the root hair

occurrence in cortical region, fungal colonization was also noticed, at random, in a few passage cells of exodermis of both the species. The mode of colonization usually began in the inner cortical cells, subsequently through re-infection, the fungal hyphae were observed in the outer cortical region. In the present study, the transverse section of *C. triplicata* showed that the pelotons occupied the entire cell cavities, particularly in the inner cortex region. Similar observations were made in *A. maculosum*. This results in the distribution of protoplasm over the surface of the hyphae. However, in the completely filled cells, the protoplasm was barely traceable (Figure 4). The pelotons were densely stained with toluidine blue O. However, the fungal hyphae extending from the 'matured' pelotons were found to be finer. The cortical zone with these 'matured' pelotons forms the digestion layer (Burgeff 1959). Unlike the digestion layer, the early stages of fungal colonization appeared to be in loose aggregate of hyphae. Similar observations were made in *Spathoglottis plicata* (Senthilkumar and

Table 2 Mycorrhizal infection of cortex tissue in *Aerides maculosum* Lindl. (epiphytic) and *Calanthe triplicata* (Willmet) Ames (terrestrial)

	Aerides maculosum		*Calanthe triplicata*	
Parameters	*Young root*	*Old root*	*Young root*	*Old root*
Mycorrhizal colonization (%)*	15.85±5.2	19.00±0.65	76.58±3.46	87.55±5.22
Undigested pelotons (%)*	94.08±0.47	94.51±0.37	91.45±0.79	95.61±0.64
Digested pelotons (%)*	5.92±0.47	5.49±0.37	8.55±0.79	4.39±0.64

*All the values were of mean percentage with standard deviation.

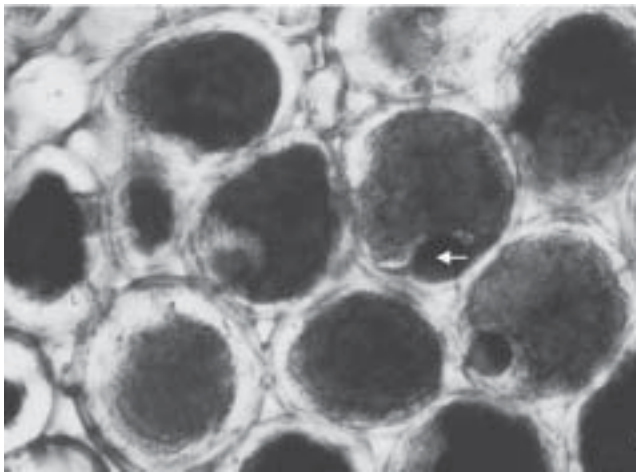


Figure 4 Transverse section of infected root showing the cortical cells packed with pelotons. The nucleus is pushed aside in the cell protoplasm (arrow)

Krishnamurthy 1998) and *Corollarhiza* (Burgeff 1959). In the present study, the cross-sections of infected roots in both *A. maculosum* and *C. triplicata* have shown some interesting cytological changes like enlargement of nuclei. This was noticed in the peloton-occupied cells, where the nuclei appeared to be present on one side in the cell protoplasm. Cytochemical study with toluidine blue O (McCully 1966) showed positive results for high DNA content. Similar cytological changes were reported earlier (Burgeff 1959; Senthilkumar and Krishnamurthy 1999; Werner 1992). The cytochemical study using coomassie brilliant blue (Fisher 1968) showed positive staining of fungal hyphae, indicating high protein content. The digestion of fungal pelotons in the cortical cells, especially in *A. maculosum*, showed the release of lipid droplets. This was positively stained by Nile blue sulphate (Cain 1968). However, the digesting pelotons in *C. triplicata* did not show the presence of lipids. The degradation of lipids and polyphosphates was observed in the degenerating hyphae of *Platanthera* (Richardson, Peterson, and Currah 1992) and in *Spathoglottis plicata* (Senthilkumar and Krishnamurthy 1998). Although, the above-mentioned observations were found to be common in both orchids, the percentage of infection density and pattern of infection differ considerably between the two species studied. The mycorrhiza, as outlined in Burgeff (1959), in both *A.* maculosum and *C. triplicata* was found to be of tolypophagy type. In this type, as explained by Burgeff (1943), the fungal mass growing on the cells of roots and rhizomes were killed as coiled aggregates and remained in the cells as clumped excretory bodies.

Although the tropical epiphytic orchids are described to be less dependent on mycotrophy due to their ready access to sunlight throughout the year (Zettler 1997), the mycorrhizal association in *A. maculosum* is perhaps an alternative nutritional requirement developed by the plant to enhance its survival (Zettler 1997). The presence of pelotons in the older roots of both *A. maculosum* and *C. triplicata* may be attributed to the fact that the orchids achieve a balance between digestion and re-infection. The results of the mean percentage of digested and undigested pelotons (Table 2) in both the species explain the fact that the host system controls the degree of peloton digestion so as to maintain the fungal association as symbiotic parasitism (Zettler 1997).

Acknowledgement

The author is highly indebted to the Joint Director, BSI, SC, Coimbatore for the facilities and guidance. The author is also highly grateful to the Director, Botanical Survey of India, Kolkata, for his constant encouragement and support.

References

Arditti J. 1966
Orchids
Scientific American **214**: 70–78

Benzing D H. 1982
Mycorrhizal infections of epiphytic orchids in southern Florida
American Orchid Society Bulletin **51**: 618–622

Burgeff H. 1943
Problematik der Mycorrhiza
Naturwiss. **31**: 358–367

Burgeff H. 1959
Mycorrhiza of orchids
In *The Orchids: a scientific survey*, pp. 361–395, edited by C L Withner
New York: Ronald Press

Cain A J. 1968
Use of Nile Blue in the examination of lipids
Quarterly Journal of Microscopic Science **88**: 383–392

Clements M A. 1988
Orchid mycorrhizal associations
Lindleyana **3**: 73–86

Currah R S and Zelmer, C. 1992
A key and notes for the genera of fungi mycorrhizal with orchids and a new species in the genus *Epulorhiza*
Reports of the Tottori Mycological Institute **30**: 43–59

Dressler R L. 1981
The Orchids: natural history and classification
Harvard: Harvard University Press

Fisher D B 1968
Protein staining of ribboned epon sections for light microscopy
Histochemie **16**: 82–96

Gahan P B. 1984
Plant Histochemistry and Cytochemistry: an introduction
Florida: Academic Press

Hadley G. 1984
Mycorrhiza in Tropical Orchids, pp. 154–159
[Proceedings of the Fifth Asean Orchid Congress Seminar, Singapore, 1–3 August 1984, organized by the Parks and Recreation Department, Ministry of National Development, Singapore]

Hadley G and Williamson B. 1972
Features of mycorrhiza infection in some Malayan Orchids
New Phytologist **71**: 1111–1118

Harley J L and Smith S E. 1983
Mycorrhizal Symbiosis
London: Academic Press

Krishnamurthy K V. 1999
Methods in Cell Wall Cytochemistry
USA: CRC Press

McCully M E. 1966
Histological studies on the genus *Fucus*. I. Light microscopy of the mature vegetative plant
Protoplasma **62**: 287–305

O'Brien T P, Feder N, and McCully M E. 1964
Polychromatic staining of plant cell walls by toluidine blue O
Protoplasma **59**: 367–373

Rasmussen H N. 1995
Terrrestrial Orchids: from seed to mycotrophic plant
Cambridge: Cambridge University Press

Richardson K A, Peterson R L, and Currah R S. 1992
Seed reserves and early symbiotic protocorm development of *Platanthera hyperborea* (Orchidaceae)
Canadian Journal of Botany **70**: 291–300

Senthilkumar S and Krishnamurthy K V. 1998
A cytochemical study on the mycorrhiza of *Spathoglottis plicata*
Biologia Plantarum **41**: 111–119

Senthilkumar S and Krishnamurthy K V. 1999
Nuclear changes in the host cells colonized by orchid mycorrhiza
Beitraege zur Biologie der Pflanzen **71**: 369–376

Smith S E and Read D J. 1997
Mycorrhizal Symbiosis, 2nd edn
San Diego: Academic Press

Weber R W S and Webster J. 2001
Teaching techniques for mycology: 14. Mycorrhizal infection of orchid seedlings in the laboratory
Mycologist **15**(2): 55–59

Werner D. 1992
Orchid mycorrhiza
In *Symbiosis of Plants and Microbes*, pp. 367–380
London: Chapman and Hall

Zettler L W. 1997
Orchid–fungal symbiosis and its value in conservation
McIlvainea **13**: 40–45

Arbuscular mycorrhizal fungi associated with *Vitex negundo* L.

Anuj Kumar Singh* and Jamaluddin**
Tropical Forest Research Institute, Jabalpur – 482021, Madhya Pradesh, India

Introduction

The association of AMF (arbuscular mycorrhizal fungi) with a number of host plants is well documented (Binu, Ashokan, and Balasundaran 2004; Gupta, Routaray, Basak, *et al.* 2003). However, the reports on their effect on growth and survival of medicinal plants are scarce (Gupta and Janardhanan 1991; Hemalatha and Selvaraj 2003; Murugan and Selvaraj 2003).

Vitex negundo L. is an important medicinal plant, commonly known as *nirgudi* or *nirgundi* in Central India. It is a large aromatic medicinal shrub (3–4 m long), inhabiting most parts of India. This well known traditional medicinal plant is astringent, cephalic, stomachic, analgesic, anti-inflammatory, anti-bacterial, and a good hair tonic. It is also used to treat scrotal swellings, arthritic pain, and rheumatic arthritis. It is used as green fencing in rural areas. It grows naturally on marginal land.

The vesicular–arbuscular mycorrhiza is a ubiquitous symbiosis in any ecosystem, probably occurring in over two-thirds of vascular plant species

*anuj_30680@yahoo.com, **jamaluddin_125@hotmail.com

(Brundrett 1991). However, so far, no effort has been made to investigate AMF association with *V. negundo,* either in their natural population or in the plantations. This study aims to investigate the AMF–*V. negundo* association in naturally occurring plant population in the campus of TFRI (Tropical Forest Research Institute), Jabalpur.

Materials and methods

In order to carry out the study, three naturally occurring sites of *V. negundo* plant population, that is, site populated with only *V. negundo* (S-1), *V. negundo* with *Lantana camara* (S-2), and *V. negundo* with grasses (S-3), were selected in the TFRI campus.

The rhizospheric soils along with fine feeder roots were collected from each site. The root samples were gently washed under tap water, softened with 10% KOH (potassium hydroxide), and stained with trypan blue following the method of Phillips and Hayman (1970). The percentage of root colonization was calculated on the basis of root segments infected with AM fungi. AM fungal spores were extracted from the rhizospheric soil using wet sieving and decanting technique of Gerdemann and Nicholson (1963). The number of AM spores present in 100 g soil was counted under dissecting binocular microscope. The identification of AM fungi was made following the manual provided by Schenck and Perez (1990). Soil pH was measured using digital (systronics 362) pH meter.

Results and discussion

Observations pertaining to AM fungal spore population and percentage of root colonization in *V. negundo* are depicted in Table 1. AMF are found to occur in the rhizosphere of *V. negundo*. Spores of eight AM fungal species belonging to three genera, namely *Glomus, Acaulospora*, and *Gigaspora*, were found to be present in the rhizosphere of this plant in its pure and mixed population with *L. camara* and grasses.

Dominance of genus *Glomus* was evident but genera *Acaulospora* and *Gigaspora* were also present in abundance. There was considerable variation in the distribution pattern of AMF in each site. Rhizospheric soil of pure *V. negundo* site (S-1) showed 490 spores per 100 g soil, 510 spores per 100 g soil in S-2, and 455 spores per 100 g soil in S-3. The percentage of root colonization in each site varied from 40% to 60%. Root samples collected from S-1 and S-2 possessed equal percentage of colonization (60%), while in S-3, only 40% of colonization was observed.

Glomus aggregatum, Glomus macrocarpum, Glomus mosseae, Glomus fasciculatum, and *Glomus intraradices* were the commonly observed species. *G. aggregatum* and *G. intraradices* were moderately present in each site. *G. fasciculatum* showed high percentage of occurrence in S-1 and S-2 while it was low in S-3. Occurrence of *G. macrocarpum* was observed to be high in S-1 while it was moderate in S-2 and S-3.

G. mosseae was the dominant AM species among all the eight AM species occurring in each site. *Acaulospora scrobiculata* also occurred in a high percentage in S-1 and S-2, while it occurred moderately in S-3. *Acaulospora delicata* showed moderate occurrence in S-1 and S-2 and low occurrence in S-3. Genus *Gigaspora* was present in each site, with high occurrence in S-1 and moderate in S-2 and S-3 (Table 2). The pH value in most of the samples collected in three sites was almost constant. The study revealed that *V. negundo* grown in marginal soil as well as in wastelands is mycorrhizal dependent. AMF possibly help in the growth and establishment of this host species.

Table 1 AMF (arbuscular mycorrhizal fungi) spore population, percentage of root colonization, and soil pH at rhizosphere of *Vitex negundo* in three different sites

Sites	*Number of AMF spores per 100 g soil*	*Percentage of root colonization* (%)	*Soil reaction* (pH)
S-1	490	60	6.3
S-2	510	60	6.5
S-3	455	40	6.4

Table 2 Percentage of occurrence of different arbuscular mycorrhizal fungi in the rhizosphere of *Vitex negundo* in three different sites

Arbuscular mycorrhizal species	*Percentage of occurrence*		
	S-1	*S-2*	*S-3*
Glomus aggregatum	35.0	40.0	38.0
Glomus macrocarpum	60.3	45.0	40.0
Glomus mosseae	85.0	78.0	75.0
Glomus fasciculatum	70.0	65.0	46.0
Glomus intraradices	48.0	45.0	42.0
Acaulospora scrobiculata	65.0	62.0	45.0
Acaulospora delicata	30.0	28.0	19.0
Gigaspora margarita	65.0	35.0	35.0

References

Binu N K, Ashokan P K, and Balasundaran M. 2004 **Association between sandal (S*antalam album* Linn.) and arbuscular mycorrhizal fungi** *Journal of Tropical Forestry* **20**(I&II): 65–70

Brundrett M C. 1991
Mycorrhizas in natural ecosystems
Advances in Ecological Research **21**: 271–313

Gerdemann J W and Nicholson 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

Gupta M L and Janardhanan K K. 1991
Mycorrhizal association of *Glomus aggregatum* with palmarosa enhances growth and biomass
Plant and Soil **131**: 261–263

Gupta N, Routaray S, Basak U C, Das P. 2003
Distribution of AM fungi in the rhizosphere of different tea clones
Mycorrhiza News **15**(1):13–18

Hemalatha M and Selvaraj T. 2003
Association of AM fungi with Indian borage (*Plectaranthus amboinicus* (Lour.) spreng) and its influence on growth and biomass production
Mycorrhiza News **15**(1): 18–21

Murugan R and Selvaraj T. 2003
Reaction of kashini (C*ichorium intybus* L.) to different native arbuscular mycorrhizal fungi
Mycorrhiza News **15**(1): 10–13

Phillips J M and Hayman D S. 1970
Improved procedure for clearing root and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection
Transactions of the British Mycological Society **55**: 158–160

Schenck N C and Perez Y. 1990
Manual for the Identification of VA Mycorrhizal Fungi
Gainesville, Florida: Fifield Hall University. 268 pp.

Response of Rangpur lime seeds inoculated with different arbuscular mycorrhizal fungi to different months of sowing on germination

P Barman, G S K Swamy, P B Patil, C P Patil
Department of Pomology, K R C College of Horticulture, Arabhavi – 591 310, Karnataka

Introduction

Germination of Rangpur lime, the important rootstock raised through seeds, varies with respect to month of sowing, as environmental factors affecting seed germination do not remain constant throughout the year. Seedling emergence is primarily a function of moisture and temperature (Finch-Savage, Phelps, and Steckel 1994). Temperature affects both seed germination percentage and germination rate (Kotowski 1926). AM (arbuscular mycorrhizal) fungi are found in symbiotic association with the citrus roots, which helps in early emergence of the seedling. On these lines, Rangpur lime seeds were inoculated with AM fungi at different months to know the effect of AM fungi and month of sowing on seed germination.

Material and methods

The present investigation was carried out at the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, during 2005/06 to study the effect of AM fungi and month of sowing on seed germination. The inoculum used consisted of a mixture of sand, soil, and FYM (farmyard manure) in 1:1:1 (v/v) proportion and root segments of maize and ragi comprising hyphae, vesicles, arbuscles, and chlamydospores of the AM fungi. AM fungal inoculum per poly bag consisted of 80–88 infective propagules per 5 g of inoculum. The design of the experiment was factorial, completely randomized block design. There were two factors: treatments (control, *Acaulospora laevis*, and *Glomus mosseae*) and different months of sowing with three replications. Observation recorded was germination percentage.

Results and discussion

The results revealed that Rangpur lime seeds treated with *G. mosseae* recorded significantly maximum germination (67.33%), while minimum germination was recorded in uninoculated control seeds (53.37%). Seeds sown in May recorded the highest germination (88.00%) followed by those sown in June (80.74%), while the lowest germination per cent was recorded in January (20.67%) and February (24.67%). With regard to interaction study, *A. laevis* inoculated seeds

Table 1 Effect of arbuscular mycorrhizal fungi and different month of sowing on germination of Rangpur lime seeds

Treatments	*Germination (%) during different months of sowing*												
	June	*July*	*August*	*September*	*October*	*November*	*December*	*January*	*February*	*March*	*April*	*May*	*Mean*
T_1 – Control	77.78	76.00	60.00	58.00	49.67	68.00	58.00	10.00	22.00	48.00	33.00	80.00	53.37
T_2 – *Acaulospora laevis*	74.44	80.00	74.00	60.00	52.00	54.00	72.00	20.00	16.00	68.00	28.00	96.00	57.87
T_3 – *Glomus mosseae*	90.00	68.00	84.00	66.00	64.00	78.00	76.00	32.00	36.00	82.00	44.00	88.00	67.33
Mean	80.74	74.67	72.67	61.33	55.22	66.67	68.67	20.67	24.67	66.00	35.00	88.00	
For comparing													
The means of				S.Em±				C.D. at 5%					
treatments (T)				1.12				3.16					
months (M)				2.25				6.32					
T × M				3.89				10.95					

sown in May recorded the highest germination (96.00%), which was on par with *G. mosseae* inoculated seeds sown in May and June (88.00% and 90.00%, respectively), and the lowest germination was recorded in control seeds sown in January (10.00%), which was on par with *A. laevis* inoculated seeds sown in January and February months (20.00% and 16.00%, respectively), indicating that month of sowing and AM fungi had significant effects on the germination of Rangpur lime seeds (Table 1).

The significant increase in germination percentage of seeds inoculated with AM fungi over control was also observed by Venkat (2004) in Rangpur lime and by Santosh (2004) and Bassanagowda (2005) in mango. The results of Allen, Moore, and Christensen (1980) also indicated that AM fungi had immense potential to produce growth-promoting substances like gibberellins along with IAA (indole-3-acetic acid), which indirectly help in better germination of seeds of *Bouteloua gracillis* over control. The differences in AM fungi in promoting germination of Rangpur lime seeds could be attributed to the differences in efficacy of AM fungi to produce growth-promoting substance like gibberellic acid. In the present investigation, seeds sown in May and June showed better germination while those sown in January and February had lower germination. This could be due to low temperature in winter months (Table 2) as low temperature reduces seed enzyme activity (Koller 1972). It also could be attributed to more production of chlamydospores in the rhizosphere of AM fungal inoculated seeds during May and June months (Kipkoriony, Fusao, Doo-Gyung, *et al.* 2002; Schubert and Cravero 1990).

In the present investigation, May and June were found to be the suitable months for highest seed germination. Among the different treatments, *G. mosseae* treatment showed better efficacy in improving seed germination.

Table 2 Meteorological data recorded during 2005/06

Month	*Rainfall* (m)	*Temperature* (°C) *Maximum*	*Minimum*	*Relative humidity* (%)
May 2005	30.5	34.1	24.2	54.93
June 2005	105.1	24.1	23.3	72.71
July 2005	146.0	27.4	22.7	83.14
August 2005	73.8	27.2	21.6	83.43
September 2005	93.8	22.1	21.4	80.72
October 2005	64.0	30.0	20.4	72.15
November 2005	66.6	29.4	15.2	64.84
December 2005	68.0	29.1	12.7	63.27
January 2006	20.0	29.5	11.8	63.23
February 2006	24.0	32.0	12.5	57.54
March 2006	21.0	33.8	17.6	59.98
April 2006	35.0	37.1	21.6	56.41
May 2006	13.1	36.6	23.1	66.38
June 2006	8.2	30.3	23.1	73.63
July 2006	4.9	27.9	22.9	77.43

References

Allen M F, Moore T S, and Christensen M. 1980
Phytohormone changes in *Bouteloua gracillis* 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
Dharwad: University of Agricultural Sciences [MSc (Hort.) thesis]

Finch-Savage, Phelps W E K, and Steckel J R A. 1994
Seedling emergence modelling and the timing of irrigation during vegetable crop establishment,
pp. 103–108
[Fourth National Symposium on Stand Establishment of Horticultural Crops, Davis, CA]

Kipkoriony L R, Fusao M, Doo-Gyung M, Kazuomi K. 2002
The relationship between cultural practices and arbuscular mycorrhizal activity in the orchards under different management systems
Journal of Japanese Society of Horticultural Science **71**(5): 601–609

Koller D. 1972
Environmental control of seed germination
In *Seed Biology*, edited by T T Kozlowski, vol. 2
New York: Academic Press

Kotowski F. 1926
Temperature relations to germination of vegetable seeds
Proceedings of American Society for Horticultural Sciences **23**: 176–184

Santosh. 2004
Enhancement of germination, growth, graft-take and stress tolerance of mango rootstocks using bioformulations
Dharwad: University of Agricultural Sciences [MSc (Hort.) thesis]

Schubert A and Cravero M C. 1990
Vesicular-arbuscular mycorrhizae of kiwi fruit in north western Italy
Acta Horticulturae **2**

Venkat. 2004
Exploitation of Rangpur lime as a rootstock for different Citrus sp.
Dharwad: University of Agricultural Sciences [MSc (Hort.) thesis]

Orchid mycorrhizal colonization in *Rhyncostylis retusa* (L.) blume

K P Radhika and B F Rodrigues
Department of Botany, Goa University, Goa – 403 206

Introduction

The family Orchidaceae is the most species-rich plant family in the world, with an estimated 17 500 to 35 000 species, and a circum global distribution (Dressler 1993). It is also one of the most advanced plant families with many adaptations that enable long-term survival. All orchids have an obligate relationship with mycorrhizal symbionts during seed germination, with most of the symbionts being *Rhizoctonia*-like fungi (Arditti 1992). Reliance on mycorrhizal interactions is an adaptive mechanism that has allowed orchids to persist in relatively less ideal habitats, and has led to their occurrence worldwide.

Understanding mycorrhizal symbiosis is of great importance, as the fungal symbionts may play a key role in determining orchid distribution and diversity. The relationship of orchids with fungi is relatively unique in the plant kingdom. Orchid species vary in their fungal specificity, both among genera and over the course of their life cycle. Orchid mycorrhizal fungi are found intracellularly in cells of the cortex, and are confined to the roots (Hadley 1982). Within the cells, the mycorrhizae form dense coils of mycelium called pelotons, which are considered as adaptations to the host cell (Hadley 1982). Ecologically, orchid mycorrhizae are important in that they allow the plant to have two possible sources of nutrition, and the plant may use fungal carbohydrates to supplement, replace or alternate with its own photosynthetic activity. Since the fungal partner is generally able to break down complex organic materials, the orchids that grow with them are able to tap unusual substrate for nutrients, including bog peat, highly calcareous soils, and dust debris or tree branches.

Rhyncostylis retusa (L.) Blume, commonly known as foxtail orchid, is a medium-sized species, found in deciduous and dry lowland forests, and savanna-like woodlands with stout, repent, short stem, curved, fleshy, ligulate, deeply channelled, and apically retuse leaves. It blooms on a 60-cm long axillary pendant called racemose, and is densely flowered (100–140), with small, magenta and white flowers (2 cm in diameter) with spots, in cylindrical inflorescence. In

the present paper, orchid mycorrhizal colonization is reported in *R. retusa*.

Methodology

Epiphytic orchid *R. retusa* (L.) Blume collected from Mollem area (North Goa) was brought to the laboratory, and velamen roots were processed for mycorrhizal colonization. The roots of the orchid plant species were washed thoroughly in water, cut into 1-cm fragments, and analysed for mycorrhizal association, using the method described by Phillips and Hayman (1970). The presence of mycorrhizal fungi in the cortical cells was examined under Leica compound microscope.

Observations and discussion

The roots of the epiphytic orchid *R. retusa* are consistently and heavily colonized by pelotons (Figure 1), representing a potentially substantial source of carbon and other nutrients. Within the cells, hyphae form coils called pelotons, which greatly increase the interfacial surface area between orchid and fungi. A membrane and an interfacial matrix material surround these pelotons (Peterson, Uetake, and Zelmer 1998). Each intracellular peloton has a short life span, lasting only a few days, before it degenerates and is digested by the orchid cell. In fact, hyphae have short life span; the older hyphae develop large vacuoles and thick cell walls, and the cytoplasm degenerates. The hyphal cells eventually collapse, and are consumed by the orchid cell. During this process, the plant cell remains functional, and can be recolonized by any surviving hyphae, or by fungi invading from adjacent cells.

Acknowledgement

The authors gratefully acknowledge the financial assistance received from the Planning Commission, Government of India, New Delhi.

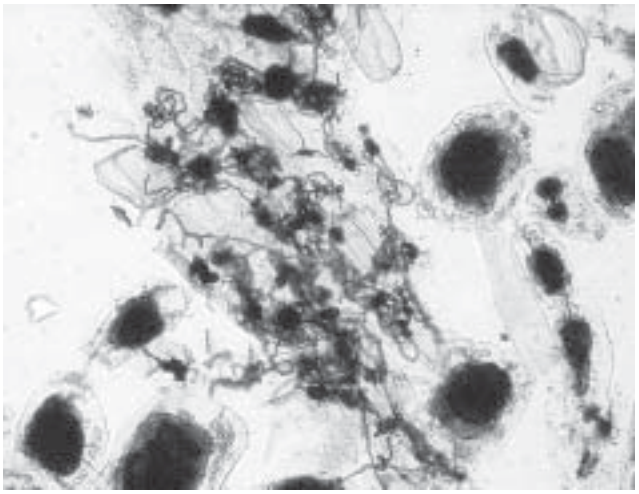


Figure 1 Orchid mycorrhizal fungi in *Rhyncostylis retusa* with pelotons in the cortical cells of the roots (400×)

References

Arditti J. 1992
Fundamentals of Orchid Biology
New York: John Wiley and Sons

Dressler R L. 1993
Phylogeny and Classification of the Orchid Family
Portland, OR: Dioscoriodes Press

Hadley G. 1982
Orchid mycorrhiza
In *Orchid Biology*: *reviews and perspectives II*, pp. 83–113, edited by A J Corness
New York: University Press

Peterson R L, Uetake Y, and Zelmer C. 1998
Fungal symbiosis with orchid protocorms
Symbiosis **25**: 29–55

Phillips J M and Hayman D S. 1970
Improved procedure for clearing root and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infections
Transactions of the British Mycological Society **55**: 158–160

New approaches

Potential of a large subunit rDNA region for phylogenetic resolution

da Silva G A, Lumini E, Maia L C, Bonfante P, Bianciotto V (2006) analysed the LSU (large subunit) rRNA (ribosomal RNA) gene (LSU rDNA [ribosomal DNA]) as a phylogenetic marker for AM (arbuscular mycorrhizal) fungal taxonomy (*Mycorrhiza* **16**(3): 183–189, 2006). Partial LSU rDNA sequences were obtained from 10 AM fungal isolates, comprising seven species, with two new primers designed for Glomeromycota LSU rDNA. The sequences, together with 58 sequences available from the databases, represented 31 AM fungal species. Neighbour joining and parsimony analyses were performed with the aim of evaluating the potential of the LSU rDNA for phylogenetic resolution. The resulting trees indicated that Archaeosporaceae is a basal group in Glomeromycota. Acaulosporaceae and Gigasporaceae belong to the same clade, while Glomeraceae is polyphyletic. The results support data obtained for the SSU (small subunit) rRNA gene, demonstrating that the LSU rRNA gene is a useful molecular marker for clarifying taxonomic and phylogenetic relationships in Glomeromycota.

Centre for Mycorrhizal Culture Collection

Bio-fertilizers in wheat–pulse rotation under agro-forestry system

Mahaveer P Sharma and Alok Adholeya
Centre for Mycorrhizal Research, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi – 110 003, India

Introduction

Field trials were conducted under a network programme on INM (integrated nutrient management) that deals with the integration of beneficial microbes and manures. These, in turn, are combined with chemical fertilizers in order to supply optimum combination of nutrients to maximize yield in wheat–pulse rotation under poplar–eucalyptus-based agro-forestry system.

Different doses of inorganic fertilizers and organic manures in combination with crop-specific bio-fertilizers such as mycorrhizal fungi, and phosphate-solubilizing and nitrogen-fixing bacteria were applied to wheat and pulse crops in marginally fertile phosphorus-deficient soil. The best combination of input in terms of inorganic fertilizers and bio-fertilizers, while achieving higher plant production and maintaining soil health with attractive cost economics, was optimized in wheat–pulse rotation.

Followings crops have been tested.

- Wheat (*Triticum aestivum* L.)
- Black gram (*Phaseolus mungo* L.)
- Green gram (*Phaseolus aureus*)

Year of trial and details on field locations

All the field trials were conducted continuously for three years. The field experiments were carried out both at TERI's field station and at a farmer's field at Badshahpur, Haryana.

Marginal wasteland (site 1)

The site is located in a semi arid zone, at Gual Pahari, Haryana, India (77° 12' E and 280° 35' N) and at 255 m above mean sea level. The site was not under any cropping pattern and productive use. The soil type was sandy loam (0–30 cm depth) hypothermic Typic Haplustalf.

Agricultural land (site 2)

The site is located in Sohna district of Haryana state and is about 50 km far from TERI laboratory. The 20% area of site was used for rearing cattle while the remaining area was available for cultivation. The site was under continuous rotation; the soil type is similar to that in case of site 1. The chemical characteristics of soil in both the sites are presented in Table 1.

Table 1 Nutrient characteristics of two experimental sites

Macro-nutrients and chemical parameters	*Site 1*™	*Site 2*
pH (1:2.5 soil:water)	7.38 ± 0.29	7.12 ± 0.13
Electrical conductivity (dS/m)	0.16 ± 0.013	0.59 ± 0.021
Available phosphorus (PPM)	3.78 ± 0.98	6.48 ± 0.91
Potassium (PPM)	92.30 ± 5.11	117.3 ± 9.30
Total nitrogen (%)	0.09 ± 0.002	0.17 ± 0.003
Organic carbon (%)	0.52 ± 0.03	0.63 ± 0.04

™ Experimental sites; site 1 – Gual Pahari; site 2 – Badshahpur farmland

How the trials were conducted?

The field trials were conducted while planting rabi and kharif crops at two different sites. The bio-fertilizers such as phosphate-solubilizing bacteria, *Rhizobium* sp., and *Azospirilium* sp. were procured from other network partners whereas mycorrhizal fungi was isolated and multiplied at Mycorrhiza Centre of TERI. Different doses including the recommended dose of NPK (nitrogen, phosphorus, potassium) fertilizers and organic manures along with crop-specific bio-fertilizers were applied to wheat and pulses (green gram and black gram) at two sites, including one farmer's field, using spit plot randomized block design. The fertilizer and manure doses tested were recommended dose, that is, 120-60-50 kg/ha (hectare) NPK + 8 tonnes/ha farmyard manure, half the recommended dose of phosphorus, that is, 120-30-50 kg/ha NPK + 8 tonnes/ha farmyard manure, 120-60-50 kg/ha NPK + 16 tonnes/ha farmyard manure and double the recommended dose that is, 240-120-100 kg/ha NPK + 8 tonnes/ha farmyard manure. Mycorrhizal inoculum procured from TERI was applied at the rate of 1000 propagules per square metre for all three crops. *Rhizobium* sp. was applied only for pulses, that is, *urad* and *moong*, by seed encapsulation. The *Azospirilium* sp. and phosphate-solubilizing bacteria were applied to wheat by seed encapsulation method.

The bio-fertilizers were inoculated continuously at each fertility dose. This way various combinations of treatment were formed. Agronomic parameters and soil nutrient status were recorded after the harvest. Based on higher grain yield and soil health, optimum fertilizer combination was evaluated for both wheat and pulses.

Findings

- Mycorrhizal application provided useful findings in terms of higher grain yield, nutrient balance, and reduced appropriate dose of chemical fertilizers. At both the farms, wheat inoculated with mycorrhiza along with other beneficial micro-organisms at double dose of organic manures (16 tonnes/ha) resulted in significantly higher production and returns compared to the double doses (240-120-100 kg/ha of NPK) of chemical fertilizers. Similarly, the pulse productivity also enhanced in *Rhizobium* sp. + mycorrhiza inoculations at double dose of organic manures.
- Overall, bio-fertilizer inoculation in wheat grown at both the locations led to 15%–25% higher yield when compared to plants grown without the application of bio-fertilizers.
- The dual inoculation of AMF (arbuscular mycorrhizal fungi) with *Rhizobium* in both green gram (*moong*) and black gram (*urad*) was found to be more beneficial than the single inoculation.
- Further, the pulse and wheat productivity also benefited from the residual impact of bio-fertilizer inoculations made during preceding crop/previous rotation. The residual effect of inoculations of preceding crop was found to be beneficial for the following crop.
- The wheat and *urad* crops benefited by previous inoculations and led to higher yield. Inoculations carried out during rabi and kharif were influenced by fertilizer doses. Rabi and kharif season crops integrated with inoculations performed better at double dose of manure compared to the chemical fertilizers.
- When the cost economics were taken into account, this point was further justified and maximum cost–benefit ratio became evident wherein bio-fertilizer inoculation was done at a particular fertility dose. The B/C ratio of bio-fertilizer and optimized fertilizer dose under agro-forestry system were more than 100% higher than the conventional system.
- Soil biological health at optimized fertility regime maintained the soil microbial activity, mycorrhizal population, and microbial count, and also maintained the nutrient profile (removal vs. gain) for sustaining the plant productivity. The improved soil health due to integration of fertilizer and manure input along with microbes was clearly evident through increment in yield and biomass.

Cost economics

Tables 2, 3, and 4 give the cost economics.

Recommendations

These findings can now form the basis for recommendations to the national network for multi-location trials, which would eventually benefit the farmer community of a region and the environment as well.

Table 2 Economics of wheat as influenced by bio-fertilizer inoculations at various fertility levels

Fertilizer levels	*Kharif inoculations*	*Current inoculations*	*Grain yield* (quintal/ha)	*Additional yield over control* (quintal/ha)	*Additional returns* (Rs/ha)	*Additional cost of input added over control* (Rs/ha)	*Additonal net returns* (Rs/ha)
120-50-40 kg	AMF	AMF	32.29	4.2	2520	450	2310
NPK AMF/ha		AZO+PSB	32.45	4.4	2640	350	2290
+8 tonnes	AMF+*Rhizobium*	AMF	33.46	5.4	3240	500	2740
FYM/acre		AZO+PSB	33.65	5.6	3360	400	2960
	Rhizobium	–	28.14	0.11	66	150	-84
		AZO+PSB	30.53	2.5	1500	250	1250
	Uninoculated	uninoculated	28.03	–	–	–	–
		AZO+PSB	28.46	0.40	240	200	40
120-50-40 kg	AMF	AMF	30.18	3.5	2100	450	1650
NPK /ha+		AZO+PSB	30.39	3.7	1920	350	1570
8 tonnes	AMF+*Rhizobium*	AMF	31.86	5.18	3108	500	2608
FYM/acre		AZO+PSB	31.66	4.98	2988	400	2588
	Rhizobium	–	26.78	0.1	600	150	450
		AZO+PSB	28.58	1.90	1140	250	890
	Uninoculated	uninoculated	26.68	–	–	–	–
		AZO+PSB	27.14	0.46	276	200	76
120-50-40 kg	AMF	AMF	36.86	6.88	4128	450	3678
NPK /ha		AZO+PSB	34.84	4.86	2916	350	2566
+16 tonnes	AMF+*Rhizobium*	AMF	36.92	6.94	4164	500	3664
FYM/acre		AZO+PSB	35.27	5.29	3174	400	2774
	Rhizobium	–	31.91	1.93	1158	150	1008
		AZO+PSB	35.41	5.43	3258	250	3008
	Uninoculated	uninoculated	29.98	–	–	–	–
		AZO+PSB	30.77	0.79	474	200	274
240-100-80 kg	AMF	AMF	36.92	5.24	3144	450	2694
NPK /ha+		AZO+PSB	35.17	3.49	2098	350	1748
8 tonnes	AMF+*Rhizobium*	AMF	36.96	5.28	3168	500	2668
FYM/acre		AZO+PSB	35.01	3.33	1998	400	1598
	Rhizobium	–	31.98	0.30	180	150	30
		AZO+PSB	34.7	3.02	1812	250	1562
	Uninoculated	uninoculated	31.68	–	–	–	–
		AZO+PSB	31.08	0.40	240	200	40

FYM – farmyard manure; AMF – arbuscular mycorrhizal fungi; PSB – phosphorus solubilizing bacteria; AZO – Azospirilium; NPK – nitrogen, phosphorus, potassium

Diammonium phosphate @ Rs 18.00/kg P; urea @ Rs 10.61/kg N; muriate of potash @ Rs 15.78/kg K; cost of PSB + Azospirilium –Rs 200/ha; cost of mycorrhiza @ Rs 300/ha; cost of Rhizobium @ Rs 100; FYM @ Rs 300/tonne; price of wheat grain @ Rs 500/quintal; price of straw @ Rs 100/quinal; control means, uninoculated at various fertility levels

Table 3 Build up (+)/depletion (–) of nutrient status due to integrated nutrient management practices in three rotations (wheat–*moong* bean-wheat; location – Gual Pahari)

Fertilizer levels	*Kharif inoculations*	*Current inoculations*	*Gain (+) / loss (–) of major nutrients in soil*		
			N	P_2O_5	K_2O
120-50-50 kg NPK /ha	AMF	AMF	0.123	6.02	14.71
+8 tonnes FYM/acre		AZO+PSB	0.129	5.94	14.37
	AMF+*Rhizobium*	AMF+*Rhizobium*	0.147	6.52	16.44
		AZO+PSB	0.146	6.03	17.41
	Rhizobium	*Rhizobium*	0.140	5.04	22.26
		AZO+PSB	0.137	5.70	24.96
	Uninoculated	uninoculated	0.113	4.51	17.86
		AZO+PSB	0.122	5.04	21.40
120-25-50 kg NPK /ha	AMF	AMF	0.165	6.72	16.70
+8 tonnes FYM/acre		AZO+PSB	0.177	6.62	15.38
	AMF+*Rhizobium*	AMF+*Rhizobium*	0.194	6.26	18.13
		AZO+PSB	0.197	6.74	17.97
	Rhizobium	Rhizobium	0.148	5.34	11.01
		AZO+PSB	0.154	6.68	12.67
	Uninoculated	uninoculated	0.136	4.22	4.63
		AZO+PSB	0.23	6.05	9.41
120-50-50 kg NPK /ha	AMF	AMF	0.174	5.79	13.10
+16 tonnes FYM/acre		AZO+PSB	0.185	6.60	12.47
	AMF+*Rhizobium*	AMF+*Rhizobium*	0.217	7.75	16.95
		AZO+PSB	0.20	7.60	16.22
	Rhizobium	*Rhizobium*	0.164	7.87	13.10
		AZO+PSB	0.170	8.72	13.22
	Uninoculated	Uninoculated	0.24	7.91	8.10
		AZO+PSB	0.163	8.75	11.82
240-100-100 kg NPK /ha	AMF	AMF	0.213	7.46	19.26
+8 tonnes FYM/acre		AZO+PSB	0.223	7.11	19.07
	AMF+*Rhizobium*	AMF+*Rhizobium*	0.234	7.60	17.43
		AZO+PSB	0.235	7.06	18.40
	Rhizobium	*Rhizobium*	0.208	6.97	20.13
		AZO+PSB	0.216	7.53	20.27
	Uninoculated	Uninoculated	0.20	5.66	16.83
		AZO+PSB	0.209	7.97	19.47

FYM - farmyard manure; AMF - arbuscular mycorrhizal fungi; PSB - phosphorus solubilizing bacteria; AZO - Azospirilium; NPK - nitrogen, phosphorus, potassium; P_2O_5 - phosphorus pentoxide; K_2O - potassium oxide

Table 4 Cost economics of wheat–pulse rotation under poplar-based agro-forestry at Gual Pahari site under integrated nutrient management trial

Treatments		*Gross returns* (Rs/year)				*Cost of cultivation/ plantation/year* (Rs)				*B/C ratio*	*B/C ratio under conventional system*
		Wheat	*Moong bean*	*Poplar*[a]	*Total*	*Wheat*	*Moong bean*	*Poplar*[b]	*Total*		
F1	Bio-fertilizer inoculation	19 800	19 420	55 000	94 220	11 216	12 512	2306	26 034	2.61	0.65
	Uninoculated	15 954	13 100	55 000	84 054	10 806	12 412	2306	25 524	2.29	0.25
F2	Biofertilizer inoculation	18 612	21 920	55 000	95 532	11 056	12 062	2306	25 424	2.76	0.75
	Uninoculated	15 072	19 100	55 000	74 115.7	10 556	11 662	2306	24 524	2.02	0.53
F3	Biofertilizer inoculation	21 312	26 620	55 000	102 932	13 006	14 012	3006	30 024	2.43	0.77
	Uninoculated	17 988	23 040	55 000	96 028	12 806	14 112	3006	29 924	2.20	0.52
F4	Biofertilizer inoculation	21 342	27 960	55 000	104 302	14 312	13 624	2306	30 242	2.45	0.76
	Uninoculated	19 008	23 500	55 000	97 508	13 812	13 424	2306	29 542	2.30	0.56

Diammonium phosphate @ Rs 18.00/kg P; urea @ Rs 10.61/kg N; muriate of potash @ Rs 15.78/kg K; cost of PSB + Azospirilium Rs 100/ ha; cost of mycorrhiza @ Rs 200/ha; cost of Rhizobium @ Rs 100; FYM @ Rs 200/tonne; price of wheat grain @ Rs 500/quintal; price of straw @ Rs 100/quintal; price of moong grain @ Rs 2000/quintal

[a] Actual cost for poplar plants in various treatments will be extrapolated after four years.

[b] Cost for poplar plantation includes cost for irrigation, effluent treatment plants, manuring, pruning, pit digging, planting, and overall maintenance; the cost of irrigation per year calculated on the basis of total cost incurred in 10 years, poplar price @ Rs 1000 per plant calculated per year based on the 10 years as gestation period/maturity.

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 no., issue no., page nos (address of the first author or of the corresponding author, marked with an asterisk)
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]
Baptista P*, Martins A, Pais M S, Tavares R M, Lino-Neto T. 2007	**Involvement of reactive oxygen species during early stages of ectomycorrhiza establishment between *Castanea sativa* and *Pisolithus tinctorius*** *Mycorrhiza* **17**(3):185–193 [Baptista P, Escola Superior Agrária de Bragança, CIMO, Apartado 1172, P-5301855 Braganca, Portugal]
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 Super Agr, Campus Sta 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 California Davis, 4348 Storer Hall, Davis, CA 95616]
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 D, University of Sheffield, Department of Animal and Plant Science, Alfred Denny Building, Western Bank, Sheffield S10 2TN, S Yorkshire, England]
Deguchi S*, Shimazaki Y, Uozumi S, Tawaraya K, Kawamoto H, Tanaka O. 2007	**White clover living mulch increases the yield of silage corn via arbuscular mycorrhizal fungus colonization.** *Plant and Soil* **291**(1–2):291–299. [Deguchi S, Natural Agricultural Research Centre, 4 Akahira, Morioka, Iwate 0200198, Japan]
Fransson P M A*, Anderson I C, Alexander I J. 2007	**Does carbon partitioning in ectomycorrhizal pine seedlings under elevated CO_2 vary with fungal species?** *Plant and Soil* **291**(1–2): 323–333 [Fransson P M A, Department of Forest Mycology and Pathology, POB 7026, S-75007 Uppsala, Sweden]

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos (address of the first author or of the corresponding author, marked with an asterisk)
Grebenc T* and Kraigher H. 2007	**Changes in the community of ectomycorrhizal fungi and increased fine root number under adult beech trees chronically fumigated with double ambient ozone concentration** *Plant Biology* **9**(2): 279–287 [Grebenc T, Slovenian Forestry Institute, Vecna Pot 2, SI-1000 Ljubljana, Slovenia]
Haberer K*, Grebenc T, Alexou M, Gessler A, Kraigher H, Rennenberg H. 2007	**Effects of long-term free-air ozone fumigation on delta N-15 and total N in *Fagus sylvatica* and associated mycorrhizal fungi.** *Plant Biology* **9**(2): 242–252. [Haberer K, University of Freiburg, Chair Tree Physiology, Institute of Forest Botany and Tree Physiology, Georges Kohler Allee 053-054, D-79110 Freiburg, Germany]
Hamel C* and Strullu D G. 2006	**Arbuscular mycorrhizal fungi in field crop production: potential and new direction** *Canadian Journal Of Plant Science* **86**(4): 941–950 [Hamel C, Agriculture and Agri-Food Canada, Semiraid Prairie Agricultural Research Centre, Box 1030 Airport Rd, Swift Current, SK S9H 3X2, Canada]
Ishida T A*, Nara K, and Hogetsu T. 2007	**Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests** *New Phytologist* **174**(2): 430–440 [Ishida T A, University of Tokyo, Grad, School of Agricultural and Life Science, Bunkyo Ku, Yayoi 1-1-1, Tokyo 1138657, Japan]
Kennedy P G and Peay K G*. 2007	**Different soil moisture conditions change the outcome of the ectomycorrhizal symbiosis between *Rhizopogon* species and *Pinus muricata*** *Plant and Soil* **291**(1–2): 155–165 [Peay K G, University of California Berkeley, Department of Environmental Science Policy and Management, 321 Koshland Hall, Berkeley, CA 94720]
Koide R T*, Courty P E, and Garbaye J. 2007	**Research perspectives on functional diversity in ectomycorrhizal fungi** *New Phytologist* **174**(2): 240–243 [Koide R T, Pennsylvania State University, Department Horticultural, University Pk, PA 16802]
Koide R T*, Shumway D L, Xu B, Sharda J N. 2007	**On temporal partitioning of a community of ectomycorrhizal fungi** *New Phytologist* **174**(2): 420–429 [Koide R T, Penn. State University, Dept Horticultural, University Pk, PA 16802]
Lilleskov E A* and Parrent J L. 2007	**Can we develop general predictive models of mycorrhizal fungal community-environment relationships?** *New Phytologist*. **174**(2): 250–256 [Lilleskov E A, US Forest Services, United States Department of Agriculture, Northern Research Station, RWU4159, 410 MacInnes Dr, Houghton, MI 49901]
Lopez M F, Manner P, Willmann A, Hampp R, Nehls U*. 2007	**Increased trehalose biosynthesis in Hartig net hyphae of ectomycorrhizas** *New Phytologist* **174**(2): 389–398 [Nehls U, University of Tubingen, Morgenstelle 1, D-72076 Tubingen, Germany]
Martin F*, Kohler A, and Duplessis S. 2007	**Living in harmony in the wood underground: ectomycorrhizal genomics** *Current Opinion in Plant Biology* **10**(2): 204–210 [Martin, F, UHP, UMR 1136, Inland Northwest Research Alliance, IFR 110, Centre INRA Nancy, F-54280 Seichamps, France]
Muleta D, Assefa F, Nemomissa S, Granhall U*. 2007	**Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia** *Forest Ecology and Management* **241**(1–3): 145–154 [Granhall U, Swedish University of Agricultural Science, Department of Microbiology, Box 7025, SE-75007 Uppsala, Sweden]
Olsrud M*, Michelsen A, and Wallander H. 2007	**Ergosterol content in ericaceous hair roots correlates with dark septate endophytes but not with ericold mycorrhizal colonization** *Soil Biology and Biochemistry* **39**(5): 1218–1221 [Olsrud M, University of Copenhagen, Department of Terrestrial Ecology, Institute of Biology, Copenhagen, Denmark]

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos (address of the first author or of the corresponding author, marked with an asterisk)
Ouahmane L, Thioulouse J, Hafidi M, Prin Y, Ducousso M, Galiana A, Plenchette C, Kisa M, Duponnois R*. 2007	**Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochtonous arbuscular mycorrhizal fungi** *Forest Ecology and Management* **241**(1–3):200–208 [Duponnois R, UCAD, Ctr Rech Bel Air, IRD, Lab Commun Microbiology, ISRA, BP 1386, Dakar, Senegal]
Rains K C* and Bledsoe C S. 2007	**Rapid uptake of N-15-ammonium and glycine-C-13, N-15 by arbuscular and ericoid mycorrhizal plants native to a Northern California coastal pygmy forest** *Soil Biology and Biochemistry* **39**(5):1078–1086 [Rains K C, Three Parameters Plus Inc, 408 Montrose Ave, Temple Terrace, FL 33617]
Rajashekar B, Samson P, Johansson T, Tunlid A*. 2007	**Evolution of nucleotide sequences and expression patterns of hydrophobin genes in the ectomycorrhizal fungus *Paxillus involutus*** *New Phytologist* **174**(2): 399–411 [Tunlid A, Lund University, Department of Microbial Ecology, Ecology Building, SE-22362 Lund, Sweden]
Robertson S J*, McGill W B, Massicotte H B, Rutherford P M. 2007	**Petroleum hydrocarbon contamination in boreal forest soils: a mycorrhizal ecosystems perspective** *Biological Reviews* **82**(2): 213–240 [Robertson S J, University of Northern British Columbia, College of Science and Management, 3333 University Way, Prince George, BC V2N 4Z9, Canada]
Sannazzaro A I, Echeverria M, Alberto E O, Ruiz O A, Menendez A B*. 2007	**Modulation of polyamine balance in *Lotus glaber* by salinity and arbuscular mycorrhiza** *Plant Physiology and Biochemistry* **45**(1): 39–46 [Menendez A B, University of Buenos Aires, DBBE, Piso 4,BAP 2,Ciudad Univ, RA-1428 Buenos Aires, DF, Argentina]
Straker C J*, Weiersbye I M, Witkowski E T F. 2007.	**Arbuscular mycorrhiza status of gold and uranium tailings and surrounding soils of South Africa's deep level gold mines: I. Root colonization and spore levels** *South African Journal of Botany* **73**(2): 218–225 [Straker C J, University of Witwatersrand, Restorat and Conservat Biol Res Grp, School of Molecular and Cellular Biology, Private Bag 3, ZA-2050 Johannesburg, South Africa]
van der Heijden M G A* and Scheublin T R. 2007.	**Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning.** *New Phytologist* **174**(2): 244–250 [van der Heijden M G A, Free University of Amsterdam, Institute of Ecological Science, de Boelelaan 1085, NL-1081 HV Amsterdam, Netherlands]
van der Putten W H*, Kowalchuk G A, Brinkman E P, Doodeman G T A, van der Kaaij R M, Kamp A F D, Menting F B J, Veenendaal E M. 2007	**Soil feedback of exotic savanna grass relates to pathogen absence and mycorrhizal selectivity** *Ecology* **88**(4):978–988 [van der Putten W H, The Netherlands Institute of Ecology, Centre for Terrestrial Ecology, NIOO KNAW, POB 40, NL-6666 ZG Heteren, The Netherlands]
Wang M Y*, Xiai R X, Wu Q S, Liu I H, Hu L M. 2007	**Influence of arbuscular mycorrhizal fungi on microbes and enzymes of soils from different cultivated densities of red clover** *Annals of Microbiology* **57**(1): 1–7 [Wang M Y, Huazhong Agricultural University, Ministry of Education, Key Lab Hort Plant Biol, Wuhan 430070, Peoples R China]
Wiemken V*. 2007	**Trehalose synthesis in ectomycorrhizas – a driving force of carbon gain for fungi?** *New Phytologist* **174**(2): 228–230 [Wiemken V, University of Basel, Institute of Botany, Zurich Basel Plant Sci Ctr, Hebelstr 1, CH-4056 Basel, Switzerland]
Zeleznik R, Hrenko M, Then C, Koch N, Grebenc T, Levanic T, Kraigher H*. 2007.	**CASIROZ: Root parameters and types of ectomycorrhiza of young beech plants exposed to different ozone and light regimes** *Plant Biology* **9**(2): 298–308 [Kraigher H, Slovenian Forestry Inst, Vecna Pot 2, Ljubljana 1000, Slovenia]

Forthcoming events

Conferences, congresses, seminars, symposia, and workshops

15–18 October 2007
Glasgow, **United Kingdom**

16th International Plant Protection Congress
Ms Louisa Simpson
BCPC Events and Conference Manager
7 Omni Business Centre, Omega Park, Alton, Hampshire, GU34 2QD, UK

Tel. +44 (0)1420 593 200 • *E-mail* louisa.simpson@bcpc.org
Fax +44 (0)1420 593 209 • *Website* http://www.bcpc.org

14–16 November 2007
Honolulu, Hawaii, **USA**

The Pacific Rim Summit on Industrial Biotechnology and Bioenergy 2007
Event Related Customer Service
Sponsorship Opportunities
E-mail pacrim@bio.org
Amanda Eden, Coordinator, Sponsorship

Tel. (202) 962-6658 • *E-mail* aeden@bio.org,
Website http://www.bio.org/pacrim/

10–15 December 2007
Cordoba, **Spain**

World Fungi 2007
Secretaría Técnica
Atril Congresos
C/Monardes, 2 local C, 41004 Sevilla

Tel. 954 226 249 • *E-mail* info@worldfungi07.es
Fax 954 221 657 • *Website* http://www.juntadeandalucia.es/medioambiente/site

16–17 January, 2008
Berlin, **Germany**

Green Week Scientific Conference 2008—Enhancing the Capacities of Agricultural Systems and Producers
Prof. Dr. H.C. Uwe Jens Nagel
Agricultural Extension and Communication Sciences
Humboldt Universität zu Berlin, Luisenstraße 53, 10099 Berlin, Germany

Tel. +49 (30) 2093 6510 • E-mail uj.nagel@agrar.hu-berlin.de
Fax +49 (30) 2093 6512 • *Website* http://www.mace-events.org/mace.html

2–6 July 2008
Krakow, **Poland**

Plant–Microbial Interactions 2008 (PMI-2008)
Katarzyna Turnau
Conference venue: Strict City Centre

E-mail pmi2008@eko.uj.edu.pl • *Website* http://www.eko.uj.edu.pl/mycorrhiza/

12–18 July 2008
Honolulu, Hawaii, **USA**

ICOM 2008: International Congress on Membranes and Membrane Processes
The North American Membrane Society
ICOM 2008 Secretariat
University of Texas at Austin, Center for Energy and Environmental Resources
10100 Burnet Road, Building 133-R7100, Austin, TX 78758

Website http://www.membranes.org

24–29 August 2008
Torino, **Italy**

ICPP 2008: 9th International Congress of Plant Pathology
Valentina Communication
Via Cibrario 27, 10143 Torino (Italy)

Fax +39 0114374318 • *E-mail* info@icpp2008.org
Website http://www.icpp2008.org

ISSN 0970-695X Regd No. 49170/89 Rs 38/-

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, I H C Complex, Lodhi Road, New Delhi – 110 003, and *printed at* Multiplexus (India), C-440, DSIDC, Narela Industrial Park, Narela, Delhi – 110 040.