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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI, The Energy and Resources Institute, was established in 1974 as the Tata Energy Research Institute. While initially, TERI's focus was mainly on documentation and information dissemination, research in the fields of energy, environment, and sustainable development was initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

The Biotechnology and Management of Bioresources Division

Focusing on ecological, environmental, and food security issues, the division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grass-roots level, with village communities. The division functions through two areas—the Centre for Mycorrhizal Research, and Plant Tissue Culture and Molecular Biology. It is actively engaged in mycorrhizal research. The Mycorrhiza Network has specifically been created to help scientists across the globe in carrying out research on mycorrhiza.

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

Established in April 1988 at TERI, New Delhi, the Mycorrhiza Network first set up the MIC (Mycorrhiza Information Centre) in the same year and the CMCC (Centre for Mycorrhizal Culture Collection) – a national germplasm bank of mycorrhizal fungi – in 1993. The general objectives of the Mycorrhiza Network are to strengthen research, encourage participation, promote information exchange, and publish the quarterly newsletter *Mycorrhiza News*.

The MIC has been primarily responsible for establishing an information network, which facilitates sharing of information among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive database on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

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

Contents

Fungal chitin and its use for estimation of mycorrhizal infection

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Fungal cell walls are characterized by the presence of chitin, cellulose, or both. The AM (arbuscular mycorrhiza) fungi are generally placed in the family of Zygomycetes as this family is characterized by the presence of chitin in cell walls. Ectomycorrhizal fungi belonging to Basidiomycetes and Ascomycetes are also known to have chitin in their cell walls. The presence of chitin can thus be used as an indicator of infection of mycorrhizal fungi in plant roots.
An assessment of the total and living biomass of the mycorrhizal fungi in plant roots is necessary in order to evaluate the degree of mycorrhization and effectiveness of mycorrhizal fungi in plants. Ergosterol, a fungal wall component, has been successfully used for assessment of living biomass of mycorrhizal fungi in mycorrhizal roots. The ergosterol estimation, however, does not indicate the fungal biomass, which has become inactive, senescent, or dead. Chitin estimation, however, indicates both living and dead/senescent fungal biomass, and it can thus be used to estimate the total biomass of the mycorrhizal fungi in mycorrhizal roots.

Detection of chitin in cell walls of mycorrhizal fungi

Studies were conducted at the Centro di Studio per la Microbiologia del Suolo, CNR, Instituto di Microbiologia Agraria, Universita di Pisa, Via del Borghetto, Pisa, Italy, to determine chemical characteristics of the hyaline outer wall of spores of the VAM (vesicular arbuscular mycorrhiza) fungi as this hyaline outer wall is of an important diagnostic value in identifying species of the AM fungi. Little is known about chemical characteristics of this outer wall. With the use of fluorochrome calcofluor and the fluorescein conjugated lectin, wheat-germ agglutinin, it was observed that chitin was one of the main components of hyaline outer wall of four species of *Glomus*. Neither chemical nor enzymatic treatments of spores were capable of changing the binding properties of this polysaccharide (Sbrana, Avio, and Giovannetti 1995).

In studies conducted at the INRA (I'institut National de la recherché agronomique), Phytoparasitologie Laboratory, Genetique et el' Amelioration Plantes Station CNRS (Centre Nationals De La Recherche Scientifique), Dijon, France, the presence of chitin was confirmed in the genera, *Glomus, Acaulospora, Gigaspora,* and *Scutellospora* in Glomales. Preliminary evidence suggested that the wall of at least one *Glomus* species possessed beta (1–3) glucan polymers. A wider range of taxa representatives of each of the four genera in Glomales was analysed for presence/absence of beta (1–3) glucan using indirect immuno-labelling with commercial monoclonal or polyclonal antibodies specific for beta (1–3) oligoglucosides. These structural polysaccharides were present in the selected *Glomus* and *Acaulospora* species but were not detected in the selected *Gigaspora* and *Scutellospora* species. The beta (1–3) glucan polymers were localized in the inner walls of the external hyphae and germ tubes, as well as in the spore walls. Endomycorrhizal fungi in Glomales have been classified as Zygomycetes, which generally have chitin and chitosan as the cell wall compounds. The distribution of beta (1–3) glucans among *Glomus* and *Acaulospora* genera suggests that the VAM fungi in the suborder Glomineae (*Glomus* and *Acaulospora*) may represent as entomophthorales, an outlying

* This paper has been compiled from TERI records in RIZA.

group in Zygomycetes, with an uncertain phylogenetic relationship. Only arbuscular fungi in Gigasporineae (*Gigaspora* and *Scutellospora*) would remain firmly placed in Zygomycetes (Gianinazzi-Pearson, Lemoine, *et al.* 1994).

In studies conducted at the Laboratoire de Biologie des Ligneux, Universite de Nancy, Cedex, France, the interface between *Tuber melanosporum* and *Corylus avellana* was studied cytochemically (PAT Ag test, Swift's reaction, and wheat germ agglutinin-colloidal gold labelling) to characterize the cell wall and matrix components. By combining the ultra-structural cytochemistry and selective extractions of polysaccharides by various solvents (EDTA, dimethyl sulfoxide, and methylamine) or enzymes (pectinase, cellulase, and cytohelicase), some ultra-structural features were made evident. The cell wall and matrix components were similar but the fungal chitin was not detected in the matrix (Pargney 1990).

Factors affecting chitin contents

Studies conducted at the Zaklad Gleboznawstwa i Nawozenia, Instytut Badaweczy Lesnictwa, Warszawa-Sekocin, Poland, on the chitin content in vegetative mycelium of Basidiomycetes and Ascomycetes mycorrhizal fungi grown in-vitro showed that the chitin content varied between species and even between strains of the given species. Also, the chitin content changed with age of the mycelium. Chitin content increased with age in the mycelium of mycorrhizal fungi belonging to Basidiomycetes while it decreased with age in the mycorrhizal fungi belonging to Ascomycetes. The N (nitrogen) source had an essential effect on the chitin content in mycelium. Less chitin content was determined in the mycelium grown on nitrate nitrogen than in that grown on urea or ammonia salts (Trzcinska and Pachlewski 1986).

In studies conducted at the Department of Soil Science and Fertilization, Forestry Research Institute, Sekocin, Poland, the effect of culture conditions on the chitin content in vegetative mycelium of the selected mycorrhizal fungi was investigated. It was proved that the chitin contents in mycelium increased as the glucose level decreased in substrate. A widening of the C : N ratio in the nutrient medium may have led to the increase of chitin level in the mycelium but still this reaction was much weaker than at the decrease of sugar concentration in substrate. It was also shown that the substrate mycelium of mycorrhizal fungi contained less chitin than the aerial mycelium. The substrate pH changes brought about by accumulation of acid metabolites during the growth did not influence the chitin level in mycelium of the mycorrhizal fungi (Trzcinska 1989).

In studies conducted at the Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden, on seasonal variation in chitin along with ergosterol and protein. Samples of ectomycorrhizal root tips were collected from a mature *Pinus sylvestris* forest on four occasions during a year (February, June, July, and October). The ectomycorrhizal roots were examined microscopically and different morphotypes were identified. Total chitin, ergosterol, and protein were extracted from each morphotype sample consisting of 25–50 mycorrhizal tips. The chitin concentration peaked in the early summer and winter sampling but remained low during the mid-summer and fall (Wallander, Massicote, and Nylund 1993).

Procedures for chitin estimation

Studies conducted at the Department of Food Science, Purdue University, W Lafayette, USA, highlighted the methods to evaluate and use chitin to detect and quantify the presence of fungi in plant and animal tissues. Chitin is a polysaccharide of beta (1–4)-linked 2-acetamido-2-deoxy-D-glucose (N-acetyl-D-glucosamine) that is found in the cell walls of fungi. In an effort to develop new methods to detect fungi in animal and plant tissues, chemical analysis based on the fungal cell wall components was evaluated. As chitin is not present in plants and most food animal tissues, the entire sample can be hydrolysed and analysed for fungal chitin. Acid, alkaline, and enzymatic hydrolyses have been used to cleave the beta (1–4) glucosidic bond to produce glucosamine, chitosan, or N-acetyl glucosamine. Major methods used to analyse these degradation products include colorimetry; chromatography (gas chromatography, high performance liquid chromatography, amino acid analysis); microscopy using fluorescent, non-fluorescent, or immuno-fluorescent dyes; near-infrared spectroscopy; and titrametric assays. Chitin has been used to estimate and quantify fungal growth in plants and plant products (Cousin 1996).

In studies conducted at the Glass House Crops Research Institute, Little Hampton, West Sussex, UK, *Laccaria laccata* and *Suillus bovinus* were grown on cellophane membranes on Petri dishes of thick, half-strength, modified Melin-Norkran's agar for up to 70 days for chitin analysis. Mycelium was then removed from the outer, middle, and inner areas of the colonies and analysed for chitin. The technique avoided problems associated with liquid cultures and provided mycelium free from agar, and this facilitated estimation of conversion factors (chitin content per unit DW [dry weight]) on different age ranges of the mycelium. Evidence of the potential of such a membrane-based growth system for providing conversion factors was provided for use in estimating the chitin content of ectomycorrhizal species and other fungi when grown on solid substrate (Whipps 1987).

In studies conducted at the Laboratoire de Recherches sur les Symbiotes des Racines, INRA (I'institut National de la recherché agronomique), Montpellier, France, on assay of fungal chitin in the

mycorrhizal roots of maritime pines, it was found that in order to estimate the fungal glucosamine resulting from hydrolysis of chitin contained in the mycorrhizal roots, it was essential to estimate the amino sugar contained in tissues of the host plant. That may be estimated by reference to a control sample or by change in the coloured reaction on the hydrolysates of mycorrhizal roots. The latter method makes it possible to determine the average quantity of these amino sugars contained in the uninfected roots of the pine and it also makes possible the direct assay of fungal glucosamine without a control sample (Vignon, Plassard, Mousain, *et al*. 1986).

In studies conducted at the Department of Forest Ecology, Section Forest Soils, Swedish University of Agricultural Sciences, Umea, Sweden, a method was developed to measure the chitin content in fungi and ectomycorrhizal roots with the HPLC (high-performance liquid chromatography). Measurements of fluorescence of FMOC-Cl (9-fluorenyl-methyl chloroformate) derivatives of glucosamine were made on acid hydrolysates of pure chitin, chitin root mixtures, and fungal root mixtures. The method was applied on five isolates of ectomycorrhizal fungi, ectomycorrhizal roots, and non-ectomycorrhizal roots of *Pinus sylvestris*. Interference from amino acids was removed by pre-treatment of samples with 0.2 N NaOH. This pre-treatment did not reduce the recovery of chitin nor did the plant material affect the recovery of chitin. The HPLC method was compared with the colorimetric chitin method by measurements on the root fungal mixtures with known fungal contents. The HPLC method gave estimates of fungal biomass equal to the expected values while the colorimetric method showed values significantly lower ($p < 0.001$) than the expected values. This chitin method offers a sensitive and specific tool for quantification of chitin in fungi and in ectomycorrhizal roots (Ekblad and Nasholm 1996).

In studies conducted at the Bentley Delivery Centre, Agriculture Research Western Australia, Locked Bag 4, Bentley, Western Australia, Australia, a method for estimation of glucosamine was based on the alkaline deacetylation of chitin to chitosan, the glucosamine residues of which were de-aminated with nitrous acid, yielding an aldehyde, which was determined colorimetrically (Shankar, Cowling, Sweetingham, *et al*. 1999).

Calculating of degree of mycorrhization by chitin assessment

Studies were conducted at the Institut National Agronomique, Algiers, Algeria, on estimation of mycorrhizal infection in Aleppo pine colonized by *Pisolithus tinctorius* and by an unknown fungus isolated from a yellow mycorrhiza on the same host. The mycelial mass was estimated from the chitin content, measured by a colorimetric method. Positive correlations were obtained between the chitin contents of roots and the number of mycorrhizae found by counting (Boughedaoui 1987).

In studies conducted at the Laboratory Recherches sur les Symbiotes des Recines, INRA, Montpellier, Cedex, France, fungal chitin assay was used to assess the degree of mycorrhization of *Pinus pinaster* roots two years after inoculation of 15-month-old plants with either *Hebeloma cylindrosporum* or indigenous micro flora, mainly comprising *Thelephora terrestris* and *L. laccata*. The fungal glucosamine (an indicator of fungal biomass) was obtained by acid hydrolysis of chitin and was estimated with reference to the control sample (tap roots) or by changes in the colorimetric reaction on mycorrhizal hydrolysates. There was a significant correlation between results obtained by these two methods. In the first method, variations in the excess glucosamine in mycorrhizal roots were studied throughout the two years of the experiment. Weight of the mycelium contained in roots was estimated after evaluating the glucosamine concentration in mycelia representative of the mycorrhizal flora (Plassard, Coll, Mousain, *et al.* 1988).

In studies conducted at the Department of Soil Science and Fertilization, Forestry Research Institute, Sekocin, Poland, the chitin content in mycorrhizal roots of pine taken from the forest nursery or cultivated in mycorrhiza synthesis in-vitro and in isolated mycorrhizae was determined. It was shown that the mycorrhizal infection could be proved on the basis of the chitin contents in roots. Biomass of the symbiont in roots would, however, be possible only if strain of the mycorrhiza-forming fungus is known (Trzcinska 1989).

In studies conducted at the Department of Forestry, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA, the chitin analysis was used to quantitatively examine the relationship between biomass of *Pisolithus tinctorius* associated with the mycorrhizal roots of *Pinus taeda* and enhanced P uptake resulting from the mycorrhizal development. The effect of P nutrition on glucosamine : fungal weight ratio was also determined; glucosamine being the proposed indicator of fungal biomass. A good correlation occurred between the total seedling P content and glucosamine contents of fine roots. Three seedlings with the lowest glucosamine values showed no visible evidence of the mycorrhizal development. The glucosamine : fungal dry weight ratio remained constant over the various environmental and cultural treatments (the P content and temperature had no obvious effects) (Rousseau, Reid, and English 1992).

Studies were conducted at the Department of Biological Sciences, Northern Illinois University, Dekalb, Illinois, USA, to calculate the intra radical fungal biomass from the per-cent colonization in the VAM mycorrhizae. Root colonization by VAM as estimated by the line intersect method yields a

quantitative value, which is related to the extent of intercellular hyphal development and the proportion of cortex occupied by cells with arbuscules. By knowing the amount of mycorrhizal colonization, it should be possible to predict the fungal volume, provided the relationship (constant) between colonization and fungal bio volume is known. Data from morphometric cytological studies of mycorrhiza of onions and chitin assay for *Agropyron smithii* roots were used to determine how the colonization and fungus bio volume are related in order to determine the constant (Kf). It was shown that by using Kf and measuring the root radius and colonized root length, the fungal bio volume could be calculated and from that; the fungal bio volume for an entire root system could be calculated. The regression model (P 0.0001) for the calculated mycorrhizal fungal biomass value (x) and the actual measured volume as determined by the chitin method (y) were good predictors of biomass (Y = 0.22 + 1.09 X; R2 = 0.89). It was thus concluded that it was possible to calculate the intra radical mycorrhizal fungal biomass from the colonization data. A preliminary Kf value of 0.06 was proposed (Toth, Miller, Jarstfer, *et al.* 1991).

Comparison of chitin with ergoterol in estimation of ectomycorrhizal infection

Studies were conducted at the United States Department of Agricultural Research Service, Washington State University, Pullman, Washington, USA, to compare the chitin and ergosterol content of 35-week-old *Pinus contorta* var. *latifolia* roots ectomycorrhiza with *Hebeloma crustuliniforme* as indices of intra metrical mycelial mass, the determination of which is necessary to quantify the effectiveness of mycorrhizal isolates in colonizing roots and enhancing the seedling performance. Preliminary laboratory trials indicated that the quantity of ergosterol, a fungus-specific membrane component, alone accounted for 77% of variability in the mycelial mass of *Hebeloma crustuliniforme* colonies. The ergosterol analysis was less time-consuming (up to four-times more samples could be prepared per day) and detected differences in the mycorrhizal establishment among the P-fertilization treatments, whereas such differences found by using chitin analysis were not statistically significant. Intra matrical mycelial estimates by the two techniques were not correlated (r = 0.15). Estimates based on the ergosterol were 2–5 times greater than those based on the chitin. The chitin–ergosterol ratio increased as the P fertilization increased. Owing to its sensitivity and specificity, it was concluded that the ergosterol assay was a practical means of monitoring the establishment of ectomycorrhizae in pine roots (Johnson and McGill 1990).

In studies conducted at the Department of Forest Ecology, Swedish University of Agricultural Science, Umea, Sweden, the chitin and ergosterol contents of ectomycorrhizal roots in three sets of experiments were studied to evaluate them as indicators of the fungal biomass. The first set of experiments showed that ageing had a marked effect on the ergosterol concentrations. The ergosterol content of seven-month-old, brown, shrunken *Pinus sylvestris–Paxillus involutus* mycorrhizae was found to be only 10% of that found in white, turgid, one- or four-month-old specimens. This supports the hypothesis that ergosterol is a good indicator of the living fungal biomass. Ageing had a lesser impact on the chitin concentration as the chitin levels found in seven-month-old mycorrhizae were still 60% of the levels found in one- and four-month-old specimens. Consequently, the chitin–ergosterol ratio increased from about 14 to 19 in one- and four-month-old mycorrhizae, respectively, to about 110 in seven-month-old mycorrhizae. In the second set of experiments, variation in the plant growth was found to have no effect on the chitin–ergosterol ratio in the whole root system of either *Alnus incana* or *Pinus sylvestris* mycorrhiza with *Paxillus involutus.* In the third set of experiments, a constant relationship was found between the two marker concentrations in ten-month root systems of *Pinus sylvestris* regardless of the fungus species involved, using *Paxillus involutus, Piloderma croceum,* and *Suillus variegatus* as test organisms. Taken together, the study suggested that both the chitin and ergosterol gave reliable but different relative measures of fungal biomass in the mycorrhizal roots. It was further demonstrated that in combination, the two chemical markers could be used to estimate both, the total and living fungal biomass derived from the ergosterol : chitin ratio (Ekblad, Wallander, and Nasholm 1998).

In another study conducted at the Department of Forest Mycology and Pathology of the above university, on seasonal variation in chitin, ergosterol and protein samples of the ectomycorrhizal root tips were collected from a mature *Pinus sylvestris* forest on four occasions during a year (February, June, July, and October). The ectomycorrhizal roots were examined microscopically and different morphotypes were identified. The total chitin, ergosterol, and protein were extracted from each morphotype (sample) consisting of 25–50 mycorrhizal tips. The objective of this study was to estimate the fungal biomass by comparing these three methods and relating these biochemical characters to the seasonal forest changes such as shoot elongation, fruit body formation, and winter dormancy. The chitin concentration peaked in the early summer and winter samplings but remained low during the mid-summer and fall. Ergosterol concentrations on the other hand, were found to remain stable throughout the seasons for each morphotype but the concentrations varied from 0.07–1.4 mg per g dry root weight between the morphotypes. A correlation between the chitin and ergosterol was found for only one morphotype (*Piloderma)*. It was thus concluded that the ergosterol was a better index of fungal biomass

as chitin varied in a manner that could not be related to the amount of biomass. Protein concentrations peaked in the winter sampling for all morphotypes and reached a minimum in fall when the N demand from the host was low. The accumulated protein pool was depleted in early summer at the time of shoot elongation (Walllander, Massicotte, and Nylund 1993).

In continuation of the above studies at the Department of Microbial Ecology, Lund University, Ecology Building, Lund, Sweden, pine ectomycorrhizae were collected from the bed rock moss and moraine sites in a mature *Pinus sylvestris* forest in the vicinity of Uppsala, Sweden, on four occasions over a period of one year. Five characteristic mycorrhizal types, namely *Piloderma,* tuberculate type, *Lactarius* like, *Russula* like, and an unidentified pink were chosen and used for further biochemical bioassays. The total protein, ergosterol, and chitin extractions were performed in sequence for each sample of the distinct mycorrhizal morphotype. Differences between sites (bed rock moss versus moraine) from which the morphotypes originated were small except for an increase in protein in *Piloderma* and tuberculate mycorrhizae on moraine sites in July and January. However, there were significant variations in the chitin and protein concentrations and in the ergosterol–chitin ratio, both between the seasons and between the mycorrhizae types. The chitin concentrations peaked in both early summer and winter for all morphotypes, significantly so for *Piloderma* and tuberculate mycorrhizae, but remained low during the mid-summer and fall. Although seasonal differences in the ergosterol concentrations were small, a significant correlation was found between chitin and ergosterol for *Piloderma* mycorrhizae. For all morphotypes, the ergosterol–chitin ratio varied seasonally from low values in January, increasing to high values in October, that is end of the season of maximum fungal growth. The small seasonal variation in ergosterol and development of ergosterol-to-chitin ratio suggested that ergosterol remained a better indicator of fungal biomass than either chitin or other available measures (Wallander, Massicotte, and Nylund 1997).

Comparison of chitin and ergosterol in estimation of vesicular arbuscular mycorrhiza infection

A greenhouse experiment was carried out at the Department of Phytopathology and Soil Microbiology, Swiss Federal Research Station, Wadenswil, Switzerland, to compare the microscopic estimation of hyphal length with chitin or ergosterol contents of *Glomus intraradices*. Red clover (*Trifolium pratense*), either inoculated or not with *G. intraradices* was grown in 30-ml plastic cylinders referred to as the IC (inoculum compartments), containing the inoculum. Bottom of the IC was removed and substituted with 40 micro m nylon net, which allowed the passage of mycorrhizal mycelia but prevented roots to pass through it. The two ICs were placed in a larger 200-ml pot filled with washed sand of particle size of 100–1000 micro m. This design provided an RFS (root-free substratum) surrounding the IC from which the VAM mycelia were collected using the EME (extraradical mycelium extraction) technique. The mycelia collected were used for chitin and ergosterol analysis. The mycelial length was also determined. Correlations were found between the two biochemical parameters (ergosterol and chitin contents) and hyphal lengths in the RFS. Mycelial lengths in the RFS averaged 4.3 m/g growth medium. The concentrations of chitin and ergosterol in the extra radical mycelium collected from the RFS averaged 0.29 micro g/m and 0.24 ng/m, respectively. Considerably higher values of both substances were obtained from colonized roots growing in the IC, averaging 5.7 milligram chitin per g root dry weight and 11.09 micro grams ergosterol per g root dry weight. This indicated that most mycorrhizal biomass was located within the root domain (Frey, Vilalrino, Schuepp, *et al.* 1994).

Distribution of chitin and other polysaccharides in cell walls of vesicular arbuscular mycorrhiza fungi

In studies conducted at the Departmento di Biologia Vegetale e Centro di Studio sulla Micologia del Terreno del CNR, viale Mattioli, Torino, Italy, distribution of N acetyl glucosamine (Gle NAc) residues in the cell wall of *Glomus versiforme* was studied by using fluorescein and gold-labelled WGA (wheat germ agglutinin). This lectin is considered to possess specific binding sites for Gle NAc and a strong affinity for its oligomers and polymers, especially chitin. The affinity techniques provide a spatial description of the occurrence of sugar residues. Chitin always occurred in the cell walls of fungus studied throughout the whole duration of its symbiotic development. Gold granules conjugated with wheat germ agglutinin were found both on the thick walls of extra radical and intra radical hyphae as well as on the thin walls of arbuscular branches irrespective of the wall structure, which was either fibrillar or amorphous. However, when fluorescin-labelled lectin was used, only the thin arbuscular branches were labelled. These observations led to the conclusion that the probe accessibility to the substrate changed on the in toto hyphae and on the thin sections, and that the thick-walled hyphae had their chitin probably hidden by the non-chitinus cell wall components. These components were soluble to the heat treatment, alkali, and oxidizing agents (Bonfonte-Fasolo, Faccio, Perotto, *et al.* 1990).

In studies conducted at the Laboratoire de Phytoparasitologie, INRA-CNRS (I'institut National de la recherché agronomique-Centre Nationals De

La Recherche Scientifique), Station de Genetique et d' Amelioration des Plantes, Dijon, Cedex, France, indirect immuno-labelling with monoclonal and polyclonal antibodies was used to investigate the distribution of Beta (1–3) glucan polymers as well as chitin in the cell walls of *Glomus mosseae* and *Acaulospora laevis* as they interacted with pea and tomato roots, respectively. The beta (1–3) glucans were detected in walls of the external hyphae, hyphal coils, and intercellular hyphae developing in the outer root tissues. The glucan component was alkali-insoluble but treatment with chitinase resulted in solubilization of most of the beta (1–3) glucans from the fungal walls. A decrease in the immuno-labelling was associated with thinning of the hyphal wall as the fungi colonized deeper in the host root, and beta (1–3) glucans could not be detected in the walls of the intercellular hyphae and arbuscules in the cortical parenchyma tissues (Lemoine, Gollotte, and Gianinazzi-Pearson 1995).

In studies conducted at the Departmento Biol Vegetale, University of Turin, Viale Mattioli 25, Turin, Italy, two monoclonal antibodies (McAbs) generated against rhamnogalacturonan 1 and characterized as specific for a terminal alpha (1–2) linked fucosyl-containing epitope (CCRC-M1) and for an arabinosylated beta (1–6) – gelactin epitope (CCRC-M7) were used in immunogold experiments to determine the distribution of the epitopes in four plants, that is, *Allium porrum* (leak), *Zea mays* (maize), *Trifolium repens* (clover), and *Nicotiana tabacum* (tobacco). These plants were chosen as representatives of both the monocots and dicots with different wall structures. Analyses were performed on the root tissues in presence or absence of the AM fungi. Differential localization of the two cell wall epitopes was found between tissues and between species, for example, in leek, the CRCS-M 1-labelled epidermal and hypodermal cells whereas the CRCS-M 7-labelled cortical cells only. Clover walls were labelled by both Mc Abs whereas maize and tobacco were only labelled by CRCS-M 7. In presence of the AM fungi, labelling was additionally found in an apoplastic compartment typical of the symbiosis (the interface) occurring around the intracellular hyphae. Epitopes binding both the McAbs were found in the interfacial material and their distribution mirrored the pattern found in the host cell wall. The finding demonstrates that composition of the interface zone in a fungus plant symbiosis reflects the composition of the wall of the host cell (Balestrini, Hahn, and Bonfonte 1996).

Studies conducted at the Departmento di Biologia Vegetale and Centro di Studio Sulla Micologia del Terreno del CNR, viale Mattioli 25 Torino, Italy, on the VAM plant interactions at the cellular level showed that cellular interactions between partners were mediated by the cell surface properties (of the cell wall and/or the plasma membranes), which play an important role during development of symbiosis. The fungal cell wall becomes thinner and changes from a fibrillar into an amorphous texture, associated with different three-dimensional arrangements of the chitin chains. Response of host cells to the fungal colonization varied in different layers of roots. Coils occurred mainly in the epidermal or outer cortical layers and did not provoke a strong host response while arbuscules colonizing the inner cortical cells stimulated cytoplasm and plasma-membrane modifications of host cells (Bonfonte-Fasolo 1987).

Distribution of chitin and other polysaccharides in cell wall of ectomycorrhizal fungi

In studies conducted at the Department of Botany, University of Guelph, Guelph, Ontario, Canada, indirect labelling of the cell wall carbohydrates by using colloidal gold conjugated with the *Lectius ulex europeus* agglutinin, wheat germ agglutinin, and concanavalin A was applied to the ectomycorrhizae synthesized in pouches between *Alnus crispa* and the basidiomycete *Alpova diplophloeus* and also to the non-mycorrhizal roots. The interface established between the two symbionts involved structural modifications of the host cell walls and the hyphal walls in the Hartig net region of the ectomycorrhiza. Significantly more binding of lectins was observed in the mycorrhizal roots than in control roots. In the Hartig net region of the mycorrhizal roots, the lectins bound intensely to the host cell walls, particularly the wall ingrowths and to the adjacent fungal cell walls, whereas, in the non-mycorrhizal roots, a sparse labelling was recorded in the cell walls. The possible explanation for this pattern of lectin binding may be that the sugar residues, L-fucose, mannose, and N-acetyl glucosamine may be utilized in synthesis of the labyrinthine wall branching of the fungus; or may be that the sugar residues are bound to a proteinaceous fraction in the host or hyphal walls; or the sugar residues bound by the lectins may be components of defence reaction elicitors released from the host walls and hyphal wall degrading enzymes. Another explanation may be that the sugar residues may simply be the result of enzymatic degradation of walls but not involving elicitors of defence reactions (Massicote, Ackerley, and Peterson 1987).

In studies conducted at the Departmento Biol Vegetale, University of Turin, Viale Mattiole, Turin, Italy, the cell wall components in ectomycorrhizae of *Corylus avellana* (hazel) and *Tuber magnatum* were investigated by using immunocytochemistry and enzyme/lectin gold techniques. Observations were made in differentiated regions of hazel roots in presence/absence of ectomycorrhizal fungus. The CBH-I (cellobiohydrolase-I) gold complex and the MAb (monoclonal antibody) CCRC-M1 revealed cellulose and xyloglucans, respectively, in the host cell walls. MAb-JIM5, which detected unesterified

pectins, labelled only the material occurring at junctions between the three cells while no labelling was found after treatment with MAb-JIM7, which detected methyl-esterified pectins. MAb CCRC-M7, which recognized an arabinosylated beta (1, 6) galactan epitope, weakly labelled the tissue sections. MAb MAC 266, which detects a carbohydrate epitope on membrane and soluble glycoproteins, labelled the wall domain adjacent to the plasma membrane. In presence of the fungus, the host walls were swollen and sometimes degraded. The labelling pattern of the uninfected tissue was maintained but abundant distribution of gold granules was found after the CBH I and JIM 5 labelling. None of the probes labelled the cementing electron dense material between the hyphae in the fungal mantle and in the Hartig net. Probes for fungal walls, that is, WGA (wheat germ agglutinin) and ConA (concanavalin A), and a polyclonal antibody revealed the presence of chitin; high mannose side chains of glycoproteins; and beta 1, 3 glucans. ConA alone led to a labelling over the triangular electron dense material suggesting that this cementing material may contain a fungal wall component (Balestrini, Hahn, and Bonfante 1996).

Effect of chitin on vesicular arbuscular mycorrhiza infection in plants

In studies conducted at the Laboratorio de Oligosacarinas, Departmento de Fiscologia Y Bioquimica Vegetal, Instituto Nacional de Ciencias Agricolas, San Jose de las Lajas, LaHabana, Cuba, seeds of tomato cultivars Manalucie, Cambell - 28 and Roms were grown in seed beds to which powdered chitin was uniformly applied (2 g /m^2 after 5, 10, and 15 days germination). Controls received no chitin. Chitin application significantly increased the seedling growth parameters as compared to controls in all three cultivars. Growth parameters and increases in them as a result of the chitin application were generally greatest in cv. Roma. The per-cent root infection with mycorrhiza was also significantly increased by the chitin application in all three cultivars (Iglesias, Gutierrez, and Fernandez 1994).

In further studies at the above Institute with tomato cultivar INCA-17, chitin was scattered over the surface of the seed bed or was mixed with seeds (1 g per kg of seeds) before sowing. Alternatively, colloidal chitin, chitin hydrolysate, or chitinosan hydrolysate was applied to soil in solution at 5 g per litre. The treatments were applied alone or in combination with *Glomus fasciculatum* inoculation. There was also an untreated control and a mycorrhiza-only treatment. Apart from chitosan hydrolysate, chitin and its derivatives increased the seedling growth parameters and mycorrhizal infection (Iglesias, Rombo, Cabrera, *et al.* 1995).

Studies were conducted at the Department of Agricultural Microbiology, Natural Research Centre, Cairo, Egypt, to determine the effect of interaction between a VAM fungus, *Glomus intraradices* No. LAP 8 and *Streptomyces coelicolor* strain 2389 on growth response, nutrition, and metabolic activities of sorghum (*Sorghum bicolour*) plants grown in non-sterilized soil amended with chitin waste over eight weeks in a greenhouse. The chitin amendment resulted in an increase in the microbial population and chitinase activity in soil. Growth of mycorrhizal and non-mycorrhizal sorghum plants increased as compared to the other treatments either in presence/absence of *S. coelicolor.* VAM inoculation significantly increased growth, photosynthetic pigments, total soluble protein, and nutrient content of sorghum plants compared to the non-mycorrhizal sorghum plants. Such increases were related to the increased mycorrhizal colonization. Inoculation with *S. coelicolor* significantly increased the intensity of mycorrhizal root colonization and arbuscular formation but the levels of mycorrhizal infection and their beneficial effects were significantly reduced with the addition of chitin waste in soil (Abdel Fattah and Mohamedin 2000).

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Research findings

Microorganisms associated with mycorrhizal fungi and their role in forest productivity

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The N_2 (nitrogen)-fixing (diazotrophic) bacteria are widely distributed in nature. Most notable among these are members of the genera *Azospirillum, Beijrinkia,* and *Darxia* (Dobereiner and Day 1976). Species of *Azospirillum*, first reported by Dobereiner and her associates, are the most common N_2-fixing bacteria associated with roots of a variety of grass (Dobereiner, Day, and Dart 1972). The occurrence of *Azospirillum* with roots of other plants has also been reported from the laboratory (Singh and Subha Rao 1979; Singh 1992). In addition to the above, prevalence of several bacteria and yeasts with some novel hosts is being briefly communicated.

Association of microorganisms with novel hosts

Vesicular arbuscular-mycorrhizal fungi

Protsenko (1975) discovered the peculiar structures in hyphae, arbuscules, and vesicles of mycorrhizal fungus in roots of pea, resembling a certain group of microorganisms, viz. prokaryotes. Mosse (1970) also observed them in spores of Endogone, forming a mycorrhiza with several plants.

Wood-decaying fungi

In 1978, Larsen and his colleagues demonstrated the nitrogenase activity measured by acetylene reduction in sporocarps of decay fungi growing on the dead tree boles. Although, he did not isolate the responsible organisms, the bacteria were detected with the scanning electron microscopy. Spano, Jurgensen, Larsen, *et al.* (1982) isolated the N_2-fixing bacteria from within a sporocarp of *Fomitopsis pinicola* (Fr.) karst growing on the decaying wood.

Sporocarps of ectomycorrhizal fungi

Isolation of bacteria from sporocarps of ectomycorrhizal fungi (Lycoperdaceae) was first reported by Swartz (1929). Recently, acetylene-reducing bacteria were isolated from within the sporocarps of the ectomycorrhizal fungi, viz. *Suillus ponderosa* Smith and Thiers, *Hymenogaster parksii* Zeller and Dodge, *Tuber melanosporum* Vitt., *Hebeloma crustuliniforme* (Bull) Quel., *Laccoria. laccata* (Scop.: Fr.) Berk Br., and *Rhizopogon vinicolor* Smith (Li and Castellano 1987). Reduction of the nitrogenase activity was attributed to *Clostridium* sp. and *Azospirillum* sp. (Li and Castellano 1987; Li and Hung 1987). The nitrogenase activity was higher with *Azospirillum* isolated from *R. vinicolor* than isolates from *H. crustuliniforme* or *L. laccata* (Li and Castellano 1987).

In my recent past visit to the Forest Science laboratory, Corvallis, USA, under the Indo–US SSP/STI programme, further attempts were made to isolate *Azospirillum* from within the sporocarps of *L. laccata, R. vinicolor,* and *H. crustuliniforme* (Courtesy Dr J M Trappe and C Y Li). Isolates from sporocarps were found to reduce acetylene even at 10 °C but not at 40 °C as compared to the *Azospirillum* of tropical origin.

Sporocarps of Agaricus bisporus (*edible mushroom*)

The prevalence of *Azospirillum, Azotobacter, Beijrinkia,* and many other non-nitrogen-fixing bacteria has also been demonstrated within the sporocarps *Agaricus bisporus* (Pal 1992; Singh and Pal 1993). These N_2-fixing bacteria were also found to enhance the root colonization and spore production of the AM fungi (Bhowmik and Singh 2004), which is attributed to the production of growth-promoting substances.

Faeces and droppings of mammals

The presence of N_2-fixing bacteria (*Azospirillum, Clostridium, Klebsiela*), *Pseudomonas* sp., actinomycetes, yeast, etc., in faeces of small inhabiting mammals has also been demonstrated by several workers. These forest mammals eat most of the fruiting bodies of the mycorrhizal fungi and these faecal pellets containing fungal spores, bacteria, and yeast get dispersed. Spores of the mycorrhizal fungi in these pellets germinate and form mycorrhizae with roots of forest trees. The faeces of these animals also contain N_2-fixing bacteria and yeast. However, the nutrient status of the faeces is equivalent to the yeast extract in promoting bacterial growth and nitrogenase activity. These organisms, including

N_2-fixing bacteria, yeasts, and spores of mycorrhizal fungi in the faeces also infect rootlets when these animals dig at the bases of a tree.

Nodules and roots of Casurina equisetifolia

Prevalence of *Azospirillum* and *Azotobacter* within roots and nodules of *C. equisetifolia* were also noted for the first time. All these bacterial isolates were gram-negative and nitrogenase-positive (Singh and Subbarao 2005).

Conclusion

These observations have indicated not only the presence of mycorrhizoshperic microorganisms but also highlighted their impact in forest rehabilitation in problematic areas as well as in forest productivity.

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Effect of arbuscular mycorrhizal fungi and other microbial inoculants on chlorophyll content of mulberry (*Morus* spp.)

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Introduction

The AM (arbuscular mycorrhizal) fungi have amply demonstrated their influence on the physiological benefits conferred by them on potential host plants (Harley and Smith 1983; Jalali 1987). The increase in stomatal behaviour and photosynthesis of host plants along with increase in chlorophyll concentration due to inoculation of the AM fungi in different plant species is well documented (Allen, Smith, Moore, *et al.* 1981; Panwar 1991; Hayman 1982). Most studies conducted till date on the various agricultural crops aim at pointing out the influence of single inoculum viz., the AM fungi on them. Mulberry, which constitutes the sole food for silkworm *Bombyx mori* L. is exclusively grown for the purpose of silkworm rearing and the quality of mulberry leaf has a direct affinity and plays a vital role in cocoon production, both qualitatively and quantitatively. Studies have proved it beyond doubt that the AM fungi also ameliorate mulberry leaf quality under a reduced fertilizer input. However, in order to know the synergistic effect of the microbial consortium on chlorophyll concentration and NPK (nitrogen–phosphorous–potassium) content of mulberry leaf under graded doses of fertilizer application, the present study was taken up.

Materials and methods

The experiment was conducted under field conditions with one-year-old, established, irrigated mulberry garden of V_1 variety situated at the Central Sericultural Research and Training Institute farm in Mysore. The experiment was laid in a randomized block design with twelve treatments and three replications each. The treatments comprised two levels of inoculation (I_0 : No inoculation and I_1 : inoculation), two sources of phosphorus (S_1: single super phosphate and S_2 : rock phosphate), and three fertilizer doses (F_1, F_2, and F_3 as recommended; 3/4th of the recommended; and ½ the recommended doses of N and P, respectively). The recommended doses of N and P are 350 kg and 140 kg/ha/y, respectively. The experimental soil type was red loam with pH of 8.29, EC 0.197 milli mhos/cm^2, organic carbon% 0.517, N % 0.0208, available P 12.90 kg/ha, available K 316.66 lbs/acre, and VAM spore load 2–3 spores/20 g soil. Plots were inoculated with a mixed culture of mycorrhiza-containing spores of *Glomus fasciculatum* and *Glomus mosseae* by intercropping technique with maize as the mycorrhizal host (Katiyar, Das, and Choudhury 1998). All plots inoculated with the AM were then subsequently inoculated with *Azotobacter* at the rate of 20 kg/ha/y and phosphate-solubilizing bacteria (*Bacillus megaterium*) at the rate of 5 kg/ha/y, phosphate-solubilizing fungi (*Aspergillus awamori*) at the rate of 5 kg/ha/y in five equal splits corresponding to the five crop harvests. The bacterial biofertilizer and fungi were used by mixing with the powdered farm yard manure and applied near the rhizosphere of mulberry by making furrows. The chemical fertilizers N and P were applied in five split doses as per the doses mentioned in the treatments above. However, K was applied at the rate of 140 kg/ha/y in the form of muriate of potash as common dose irrespective of treatments. Fertilizers were applied after a gap of 10–12 days of the application of biofertilizers. The chlorophyll content of mulberry leaf was assessed following dimethyl sulphoxide method (Ronen and Galun 1984). Besides, the effect of co-inoculation on the NPK content of mulberry leaf was also estimated by (Jackson 1973).

Results and discussion

The analysis of chlorophyll a, b, and total chlorophyll content of leaf revealed a significant variation due to the different treatments (Table 1). Both, chlorophyll-a, chlorophyll-b, and the total chlorophyll of leaf were highly influenced due to co-inoculation of mulberry with different microorganisms. Significantly higher amounts of chlorophyll-a (2.257 mg/g dry weight), chlorophyll-b (0.671 mg/g dry weight), and total chlorophyll (2.928 mg/g dry weight) were recorded in the treatment T_8 as compared to rest of the treatments, including T_0 as control. The increased chlorophyll content must have been a result of enhanced stomatal conductance, photosynthesis, and transpiration (Levi and Krikun 1980; Hayman 1983) coupled with effective synergism of various microbial inoculants.

A perusal of the first year data revealed a significant variation in the N, P, and K contents of the leaf due to different treatments (Table 2). The highest leaf NPK contents (3.85%, 0.46%, and 1.83%, respectively) were recorded in the treatment T_8, which was significantly higher than rest of the treatments. However, the treatment T_8 was found to be at par with the treatments T_0 and T_4 in respect of P, and T_0 in respect of K. A similar result was also obtained in the second year in respect of leaf the NPK. Significantly higher leaf NPK

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Table I Effect of co-inoculation of vesicular arbuscular mycorrhiza phosphate solubilizing microorganisms and nitrogen-fixing bacteria on chlorophyll content of mulberry

	Leaf chlorophyll content (mg/g dry leaf wt)		
Treatment	*Chlorophyll a*	*Chlorophyll b*	*Total chlorophyll*
T_0	2.250	0.581	2.831
T_1	1.988	0.380	2.368
T_2	1.960	0.394	2.354
T_3	1.968	0.413	2.381
T_4	1.966	0.581	2.547
T_5	2.083	0.517	2.600
T_6	2.028	0.425	2.263
T_7	1.961	0.430	2.391
T_8	2.257	0.671	2.928
T_9	2.056	0.489	2.545
T_{10}	1.972	0.410	2.382
T_{11}	1.969	0.349	2.318
Critical difference at 5%	0.078	0.159	0.175

contents of 3.89%, 0.49%, and 1.92%, respectively, were observed with the treatment T_8 as compared to rest of the treatments except T_0 in respect of the leaf K. A significantly higher root colonization by the AM fungi due to co-inoculation of mulberry and application of reduced dose of N and P also justifies the above statement as the increased root colonization is an indication of better association of the AM fungi in the rhizosphere of mulberry (Fathima, Das, and Katiyar 2000), which might have increased mobilization of various nutrients, including P. The AM fungi not only help plants in better absorption of P but also help indirectly in increased uptake of other macro and micro nutrients (Safir, Boyer, and Gerdemann 1972). Reports are also available that the AM fungi in association with the N-fixing bacteria, can increase the N-fixing capacity of many crop plants, including legumes (Patterson, Chet, and Kapulnik 1990).

Table 2 Effect of co-inoculation of vesicular arbuscular mycorrhiza phosphate-solubilizing microorganisms and nitrogen-fixing bacteria on NPK content of mulberry leaf

Treatment	*Leaf nitrogen (%) (I year) (II year)*	*Leaf phosphorus (%) (I year) (II year)*	*Leaf potassium (%) (I year) (II year)*
T_0	3.71 3.88 (2.053) (2.094)	0.45 0.48 (0.974) (0.994)	1.82 1.92 (1.525) (1.557)
T_1	3.02 3.04 (1.877) (1.976)	0.36 0.42 (0.927) (0.961)	1.52 1.70 (1.423) (1.485)
T_2	3.06 3.49 (1.884) (1.998)	0.37 0.42 (0.932) (0.961)	1.58 1.66 (1.442) (1.472)
T_3	3.07 3.58 (1.890) (2.021)	0.37 0.41 (0.936) (0.957)	1.54 1.56 (1.430) (1.436)
T_4	3.41 3.84 (1.979) (2.093)	0.45 0.45 (0.974) (0.978)	1.77 1.80 (1.507) (1.517)
T_5	3.61 3.88 (2.028) (2.094)	0.44 0.48 (0.969) (0.994)	1.75 1.86 (1.501) (1.537)
T_6	3.51 3.71 (2.003) (2.053)	0.42 0.48 (0.960) (0.994)	1.75 1.84 (1.501) (1.530)
T_7	3.23 3.51 (1.933) (2.004)	0.43 0.43 (0.966) (0.964)	1.73 1.77 (1.495) (1.506)
T_8	3.85 3.89 (2.087) (2.095)	0.46 0.49 (0.983) (0.995)	1.83 1.92 (1.526) (1.556)
T_9	3.18 3.85 (1.918) (2.087)	0.37 0.46 (0.932) (0.979)	1.67 1.73 (1.475) (1.493)
T_{10}	3.04 3.16 (1.883) (1.914)	0.36 0.38 (0.927) (0.942)	1.27 1.55 (1.330) (1.434)
T_{11}	3.24 3.27 (1.933) (1.943)	0.36 0.41 (0.927) (0.957)	1.22 1.28 (1.311) (1.335)
Critical difference at 5%	0.01 3 0.029	0.012 0.003	0.012 0.017

Treatment details are same for both Tables I and II.

Treatment details

T_0:$F_1S_1I_0$	T_1:F_1S_2	I_0T_2:$F_2S_1I_0$
T_3:$F_2S_2I_0$	T_4:$F_1S_1I_1T_5$	:$F_2S_1I_1$
T_6:$F_3S_1I_1$	T_7:$F_3S_2I_1$	T_8:$F_2S_2I_1$
T_9:$F_1S_2I_1$	T_{10}: $F_3S_2I_0$	T_{11}: $F_3S_1I_0$

F_1: Recommended dose of nitrogen and phosphorus (350:140:140 kg/ha/y)
F_2: 3/4th of recommended dose of nitrogen and phosphorus
F_3: ½ of recommended dose of nitrogen and phosphorus
I_0: No inoculation
I_1: Inoculation
S_1: SSP (single super phosphate)
S_2: RP (rock phosphate)

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New approaches

Use of radiocarbon to determine mycorrhizal status of fungi

Radiocarbon was used by Hobbie E A, Weber N S, Trappe J M, vanKlinken G J (2002) to determine the mycorrhizal status of various fungi (*New Phytologist* **156**[1]: 129–136). Measurements of C^{13} in the fungal sporocarps are useful in assessing the mycorrhizal or saprophytic status of fungi. Because C^{14} measurements can indicate the age of fungal C (carbon) and mycorrhizal fungi depend closely on the recent photosynthates, C^{14} may provide an additional insight into the possible

mycorrhizal status. Sporocarps, needles, and litter from Woods Creek, Oregon, USA, together with archived sporocarps were measured for C^{14} content by accelerator mass spectrometry. Known mycorrhizal fungi resembled the current year needles (Amanita, Cantharellus, and Gomphidius) or atmospheric CO_2 (Tuber) in C^{14} and indicated an average age of 0–2 years for the incorporated C whereas saprophytic genera (Pleurocybella, Lepiota, and Hypholoma) were composed of C at least ten years old. Of genera tentatively considered as mycorrhizal from the previous work with C^{13}, only Otidia and Sowerbyella appeared mycorrhizal from C^{14} measurements whereas Aleuria, Clavulina, Paurocotylis, and Ramaria (sensu lato) consisted of older carbon and were presumably saprophytic. The C^{14} clearly separated the known mycorrhizal or saprophytic fungi and indicated C^{13} measurements should therefore be interpreted cautiously on species of unknown status. The C^{14} results for needles and mycorrhizal fungi suggested that the C sources other than atmospheric CO_2 may contribute the small amount of C. The possible sources include storage of carbohydrates and amino acids, organic nitrogen uptake, and incorporation of soil-respired CO_2 by anaplerotic or photosynthetic pathways.

Centre for Mycorrhizal Culture Collection

Root organ culture of arbuscular mycorrhizal fungi: step towards reaching sustainable agriculture

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Introduction

The AM (arbuscular mycorrhizal) fungi being obligate biotrophs had limitation in the bulk propagation in isolation from host roots. These are conventionally propagated using pot-based methods with host trap plants. The disadvantage of this mode is the low recovery of mycorrhizal propagules and contamination by saprobes, pathogens, and other mycorrhizal fungi because of improper management techniques and long-gap duration between the set-up and harvest. Several alternatives to this mode have been designed but in all, the current methodologies of cultivating the AM fungi, host plant is indispensable. It is well-known that these biofertilizer organisms are broad-spectrum and non-specific. A single species is known to colonize approximately, 90% of the land plants. These biofertilizers have a broad ecological adaptability and are known to occur in deserts as well as the arctic, temperate, tropical, and other habitats. They are also known to offer a 25%–50% reduction in phosphorus fertilizer application depending upon the plant (Tiwari, Prakash, and Adholeya 2002). However, the major bottleneck of bulk production exists towards its maximum utilization. With this limitation, the potential of this widely accepted plant beneficial fungal group was not fully exploited to reach the end users. A major breakthrough was achieved with the pioneering work of Mosse and Hepper (1975) followed by Strullu and Romand (1987) and Becard and Fortin in 1988 by proposing a new methodology for the AMF propagation by the monoxenic cultivation system. This mode of propagation exploited genetically engineered transformed roots of the potential host plants by using Ri R-T DNA of *Agrobacterium rhizogenes* by the transformation technique proposed by Tepfer (1989). Since then, till date, several species of the AMF have been successfully cultivated as root organs (Fortin, Becard, Declerck, *et al.* 2002).

Monoxenic cultivation has several advantages over conventional pot cultivation systems regarding inoculum production. This technique offers pure, sterile, bulk, contamination-free propagules, which otherwise are not achievable by using conventional modes of pot culture or aeroponic/ hydroponic techniques. In addition, this technique has an edge over other conventional modes of mass production wherein a several-fold increase in spore/propagule production is achieved in less time and space.

Further refinement of several rate-limiting factors leads to development of the technology, which involves the extraction of potential viable propagules from soils, surface sterilization, and optimization of growth conditions for germination under aseptic conditions. This is followed by association of propagules with a suitable excised host root for continuity of the system and bulk recovery of propagules. Mass-produced propagules are then formulated in autilizable form and stored before application to the target plant.

Formulation development

As the produce through in vitro mode is very concentrated as compared to the conventional trap culture technique, it is essential to develop appropriate formulations. Several mycorrhizal inoculum formulations have been proposed so far using various sources of mycorrhizal inoculum. Redecker, Thierfelder, and Werner (1995) proposed the glass beads at the research laboratory level, Plenchette, Furlan, and Fortin (1983) proposed expanded clay for the commercial sector. These formulations have the advantages to allow the natural entrapment of mycorrhizal spores and roots during the growth phase under greenhouse conditions. Beads have a porous texture with numerous air spaces into which the mycorrhizal propagules fit well. Mixing of the air-dried inoculum with inert carriers such as sand, vermiculite, and soil-rite has also been documented (Millner and Kitt 1992). Mycorrhizal inoculum is available in the form of powdered inoculum, tablets/pellets or granules, gel beads and balls, etc.

The process of mass production when adopted at the industrial level needs to be cost economic. Under this condition, where each step in the production process adds to the cost of the end product, carrier cost is an important criterion in the process development. A successful formulation carrier must be economically viable to produce (preferably made of a locally available inert material with non-toxic waste) with no deleterious effects on the mycorrhizal symbiosis and it should also be easy to handle, allowing effective dispersion during application. The formulation should permit early dissolution or dispersion (for tablets/pellets/granules) near the roots in case of potted plants so that roots can easily invade the carrier for efficient mycorrhization.

The conditions of inoculum production are as crucial as a living organism. This implies that the viability and intactness of organisms cannot be compromised at any stage of the process, beginning with incubation, monitoring of development and possible contamination, harvesting, drying, or grinding till the organism is finally mixed with the carrier and packaged. Strictly controlled growth conditions must be applied with careful attention paid to maintaining the effectiveness of the inoculum.

Economics at industrial level

Different costing parameters, such as manpower requirement, infrastructural requirements, and time and chemical requirements need to be accounted for enumeration of results for its practical feasibility. A comparative cost economics would be desirable to get a better understanding of individual components and the functional aspects involved in the system for its scale-up (Verma and Adholeya 1996). While considering the possibility of scale-up in the given infrastructure, it would be useful to consider the system that is most economical and can provide maximum units of inoculum within the hardware support available.

Application strategy

In principle, symbiosis can be achieved from only one propagule that germinates and infects a host root but it may take a long time for the AM fungus to spread to a significant portion of the root system under such conditions. Therefore, it is better to initiate multiple infections to speed up the colonization process as shown by the infectivity assays of the inocula (Sharma, Gaur, Bhatia, *et al.* 1996). Typically, one refers to the number of fungal propagules delivered by each product onto each seed or into the soil around each seed. In theory, the larger the number of AM fungal propagules delivered to the root zone at application, the faster the colonization of roots. Several methods have been proposed for its inoculum. These include broad-casting in furrow application, seed coating, root dipping, and seedling inoculation.

Quality parameters for AMF

For the AMF mass production, critical benchmarks at all stages of inoculum development, covering all possible parameters desirable for ensured desirable production need to be critically defined (Adholeya, Tiwari, and Singh 2005). These include assurance on viability at each processing step till the formulation stage, ranging from colonization of production host roots, propagule estimations, infectivity potential of crude and formulated diluted inoculum, and formulation conditions such as temperature and suitable storage conditions. Such benchmarks also help the institutionalized process efficiency at the production level.

Towards organic cultivation

Organic farming severely restricts the use of artificial chemical fertilizers and pesticides. Instead, it relies on developing a healthy, fertile soil and growing a mixture of crops. Supplementing the nutrient requirement of crops through organic composts/manures is essential for sustaining soil fertility and crop production. Mycorrhiza in

combination with other beneficial microorganisms has a promising option to contribute in organic cultivation in a drive towards minimizing excessive chemical usage.

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Biography of Prof. Bhavdish Johri

Prof. Bhavdish Johri has been actively associated with the plant–microbe interaction research for over two decades wherein the AM (arbuscular mycorrhiza) fungi and the PGPR (plant growth-promoting rhizobacteria) have been studied as independent and interacting partners. Born in 1945 in Uttar Pradesh, India, Prof. Johri obtained his MSc from Agra University in 1965 and PhD from the University of Alberta, Edmonton, Canada, in 1969. He has recently retired from the G B Pant University of Agriculture & Technology where he established a school of Microbial Diversity and Molecular Microbial Ecology, and has now moved on to become the Visiting Professor to Barkatullah University, Bhopal. Prof. Johri was instrumental in developing a status paper on Mycorrhizal Research in India that led to the first network support on this subject by the Department of Biotechnology. One of the fallouts of this effort was creation of the Centre for Mycorrhizal Culture Collection at TERI. As chairperson of the DBT Task Force on Integrated Nutrient Management, Prof. Johri was instrumental in the initiation

of a network programme on the INM (integrated nutrient management) across the various agro-ecological regions of the country wherein the mycorrhizae were a key component. A programme on transgenic biofertilizer was also organized by him for the DBT support during this period. He is currently associated with the various scientific committees of INSA (Indian National Science Academy), the DBT (Department of Biotechnology), the DST (Department of Science and Technology), NAAS (National Academy of Agricultural Sciences), and the MoEF (Ministry of Environment and Forests), and was instrumental in coordinating a 'Microorganism Diversity: strategy & action plan' on behalf of the MoEF, Government of India.

As a Guest Editor for *Current Science*, he has coordinated the preparation of a Special Section on Microbial Diversity, which carries the status of mycorrhizal research in India as a component of fungal diversity. He is a Fellow, National Academy of Sciences, India, and National Academy of Agricultural Sciences, India. Some important contributions of his research group are highlighted below.

Degraded ecosystem and stress alleviation

As a consequence of extreme stress under the degraded ecosystem, the use of *Glomus caledonicum* was found to improve resistance of the host plant through accumulation of proline, and higher rates of photorespiration of the VAM and P-amended stressed plants. This study in particular, showed that in the water-stressed maize, the net assimilation rate increased significantly only at a low water potential. This work, presented at the *Mycorrhiza Conference at HAU* (1990) by B Ramakrishnan, a Master's student, earned him a gold medal.

Zinc uptake kinetics

Soils of the Tarai region are deficient in Zn^{2+}, therefore, the role of VAM in its uptake was evaluated in an in vitro *Glomus macrocarpum*, *Zea mays* system employing $^{65}Zn^{2+}$. A detailed kinetic study showed operation of five, concentration-dependent phases of Zn^{3+} absorption, which were carrier-mediated. When validated in a legume system (*Phaseolus vulgari*s L.), a higher number of carrier sites appeared responsible for greater uptake in the mycorrhizal French bean at lower Zn^{2+} concentration.

Growth and nutrient uptake in eucalyptus hybrid

In view of utilization of *Eucalyptus* as a preferred tree species in afforestation programmes, improved seedling establishment was attempted employing fluorescent *Pseudomonas* PRS9 and indigenous AM consortium. An interactive study of the two partners in a dose-dependent manner under the varying regimes showed that quality index of the seedlings without the AM treatment was significantly higher and this was independent of the soil P levels. Irrespective of treatments, the AM inoculation efficiency was superior at 10 parts per million P. Also, an AM dose of approximately, 400 spores per seedling significantly improved the inoculation efficiency of *E. hybrid.* However, dual inoculation of the AM+PRS9 inhibited both, the growth promotion and nutrient uptake.

Host genotype-dependent impact of soil phosphorous

In a study of maize genotypes of the varying P requirement from low to high P, different growth response of a native AM consortium in sandy loam was observed. At 90 mg P and 120 mg P/kg soil, a growth depression in roots and shoots of the AM plants was observed for three of the four maize genotypes. Such depression was not observed in the non-mycorrhizal plants. While the root and shoot P and shoot Zn^{2+} concentrations were significantly greater in the AM compared to non-AM plants, the pattern of P and Zn^{2+} uptake in both the plant groups was genotype-specific. Alkaline phosphatase activity in the AM and non-AM roots was variable with respect to both, genotype and soil P. It thus, appeared that in maize, the host genotype determined the impact of soil P on the AM symbiosis.

Reciprocal AM–PGPR interaction

In view of the changed scenario of sustainable production systems and likely introduction of PGPR (plant growth-promoting rhizobacteria), such as bacilli and pseudomonads in agro-ecosystems, their capacity to secrete antifungals is a cause of concern for the natural symbionts such as mycorrhiza and rhizobia. Any negative influence of an introduced bioinoculant in an agro-ecosystem could become counter-productive. In a study of the pseudomonads in our group from wheat rhizosphere, a gene pool of over 200 isolates was subject to colony hybridization to detect the presence of Phl D gene, responsible for production of 2, 4-diacetylphloroglucinol. Further confirmation of phl D gene was carried out using specific primers and a two-step PCR, followed by in vitro production of DAPG (diacetyl phloroglucinol) and confirmation by the HPLC (high-performance liquid chromatography). All six phl D^+ isolates were able to secrete amylase and cellulase, and three were positive for pectinase. Interaction of a consortium of the AMF with phl D^+ bacterial population in soil microcosm resulted in a positive response. Interestingly, population of the culturable bacteria remained unaffected by inoculation of the AM fungi, both in the rhizosphere and rhizoplane/endorhizosphere fractions. With some of the PGPR isolates, there was an increased AM colonization and production of prominent vesicles, suggesting 'mycorrhization helper' action. That

bacterial consortia of the DAPG and phenazine producers could exert a negative influence on the AM colonization and P-accumulation has also been observed. This information derived from a joint effort in association with researchers from TERI and Swiss Groups at Basle and Neuchatel, has helped in selection of appropriate bacterial inoculants for the wheat agro-ecosystem.

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.

- *Applied Soil Ecology*
- *BMC Microbiology*
- *European Journal of Horticultural Science*
- *Forest Science*
- *Global Change Biology*
- *Journal of Basic Microbiology*
- *Molecular Plant-Microbe Interactions*
- *Mycorrhiza*
- *Mycologia*
- *New Phytologist*
- *Pedobiologia*
- *Plant Cell and Environment*
- *Plant and Soil*
- *Soil Biology & Biochemistry*

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)
Andre S, Galiana A, Le Roux C, Prin Y, Neyra M, Duponnois R.* 2005	**Ectomycorrhizal symbiosis enhanced the efficiency of inoculation with two Bradyrhizobium strains and *Acacia holosericea* growth** *Mycorrhiza* **15**(5): 357–364 [IRD UR 083 Interact Biol Sols Syst Anthropises Tr, BP 182, Ouagadougou, Burkina Faso]
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Forthcoming events

Conferences, congresses, seminars, symposia, and workshops

Chhattisgarh, **India**
26–28 October 2005

International Conference on Plant Genomics & Biotechnology: challenges & opportunities
Dr Sanjay K Katiyar, Organizing Secretary, Indira Gandhi Agricultural University, Raipur – 492 006, Chhattisgarh, India

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International Symposium on Frontiers in Genetics and Biotechnology: retrospect and prospect
Prof. P B Kavi Kishor, Convenor, International Symposium, Department of Genetics, Osmania University, Hyderabad – 500 007, India

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