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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI was established in 1974. While in the initial period the focus was mainly on documentation and information dissemination activities, research activities in the fields of energy, environment, and sustainable development were 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 Bioresources and Biotechnology 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 grassroots level, with village communities. The Division functions through five areas—Centre for Mycorrhizal Research, Microbial Biotechnology, Plant Molecular Biology, Plant Tissue Culture, and Forestry/Biodiversity. The Division 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 information sharing among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive databases 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/handling.

Contents

Role of mycorrhiza in tree plantings in the field, Part II: field inoculation, fungal succession, and effect of climatic and edaphic factors

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Field inoculation

Inoculation with ectomycorrhizal fungi

Studies conducted at the Nauchnye Doklady Vysshei, Biologicheskia, Nauki, Russia, on *Pinus cretacea* (*P. sylvestris* var. *cretacea*), raised by direct sowing from seeds, inoculated or not inoculated with mycorrhiza, on steep chalky slopes with or without site preparation, showed that inoculation with mycorrhiza had a clear advantage, especially when coupled with the site preparation treatments (surface soil loosening, weeding, watering, etc). Non-inoculated seeds produced few seedlings, many of which died at the end of the first growing season. Inoculated seeds with site preparation treatments gave good germination (Bedenko 1988).

Studies conducted at the Department of Forest Science, Oregon State University, Corvallis, USA, on conifer seedlings raised on old (8–27 years), unsuccessfully reforested clear-cut sites showed that transfer of soil from a plantation raised on a previously burnt clear-cut site at an elevation of 1720 m increased the survival of first year Douglas fir seedlings by 50%, doubled mycorrhiza formation, and tripled basal area growth. Soil from mature forests did not improve survival and growth. On the other two sites at lower elevations (1005 and 500 m), less dramatic effects owing to soil transfer were evident. It is thus evident that decline in populations of mycorrhizal fungi and perhaps other beneficial soil biota due to delay in reforestation or due to absence of other host plants can be offset by soil transfer from vigourous young plantations (Amaranthus and Perry 1987).

In further studies conducted at this university, about 150 ml soil from an established Douglas fir plantation was added to planting holes when Douglas fir seedlings was planted on an old, unrevegetated clear-cut sites. Six weeks after planting, seedlings receiving plantation soil had formed 62% more root tips than controls, had. After 15 weeks, however, no statistically significant differences were apparent. By that time, a small percentage of root tips was visibly mycorrhizal; seedlings receiving the transferred soil had the most colonization (13.6 vs. 3.5 per seedling in control, phosphorus being less than or equal to 0.05). Of seedlings receiving transferred soil, 35.6% survived during first growing season compared to 11.3% survival in control seedlings. This increase in survival rate with transferred soil is significant, especially at the place of the experiment where growing period is very small (Amaranthus and Perry 1989a).

In further studies at the university, one-year-old non-mycorrhizal Douglas fir (*Pseudotsuga menziesii*) seedlings were planted in 1985 in clear-cut blocks within the three adjacent vegetation types, namely, white leaf manzanita (*Arctostaphylos viscida*), annual grass meadow, and an open stand of white oak (*Quercus garryana*). Pasteurized or unpasteurized soil from a nearby Pacific madrone (*Arbutus menziesii*) stand was transferred to the planting holes of the seedlings in subplots within each block; control seedlings received non-madrone soil. Second-year survival averaged 92%, 43%, and 12% for seedlings planted at manzanita, meadow, and oak sites respectively. Growth differences generally paralleled survival differences. Addition of madrone soil, pasteurized or unpasteurized, did not influence survival, but unpasteurized madrone soil substantially increased growth of seedlings and nearly tripled the number of mycorrrhizal root tips of seedlings on the manzanita site but not on meadow or oak sites. Pasteurized madrone soil did not exert any influence on any site (Amaranthus and Perry 1989b).

* Compiled from TERI database – Riza

Inoculation with VA mycorrhizal fungi

Studies conducted at the Department of Plant Pathology, University of California, showed that broadcasting, banding, and drilling were more effective in field inoculation of plants with the VAM fungus *Glomus deserticola* than in seed inoculation or the application of lyophilized roots in both direct seeded and transplanted citrus seedlings. Mechanized field inoculation of direct seeded and transplanted citrus seedlings is feasible using fertilizer banding equipment and seedling machines. Inoculum remained consistently infective after soil inoculation for upto 2.5 months in fumigated field soil and for upto 1.5 months in mycorrhizal pot cultures of citrus grown in the greenhouse (Ferguson and Menge 1986).

Succession in mycorrhizal fungi in field plantings

Persistence of nursery inoculated fungi in the field plantings

Studies conducted at the Department of Forest Science, Oregen, USA, on container-grown Douglas fir seedlings inoculated with commercially grown vegetative inoculum of ectomycorrhizal fungi showed that after transplantation and growth in nursery beds for 17 months, the mean new short-root colonization of all seedlings was 80%. *Hebeloma crustuliniforme*, which formed mycorrhiza on 90% of short roots after 4.5 months in a container nursery, persisted as a dominant mycorrhizal fungus after transplantation of seedlings initially inoculated with this fungus. *Laccaria laccata* inoculated seedlings, which developed mycorrhiza on 83% of short roots after 4.5 months in the container phase, had 40% colonization of short roots after transplanting with another 40% colonized by native fungi, *Rhizopogon* spp. and *Thelephora* spp. All mycorrhizae of control seedlings and those inoculated with *Pisolithus tinctorius* were formed by fungi native to nursery beds. At the nursery stage also, *Laccaria laccata* or *Hebeloma crustuliniforme* inoculated seedlings had significantly more mycorrhizal and total short roots than *Pisolithus tinctorius* (with only four mycorrhizal root tips) or uninoculated control seedlings did. No significant differences were detected in seedling growth at the end of the container phase, but after transplantation, control seedlings were significantly taller than *L. laccata* inoculated seedlings (Hung and Trappe 1987).

In studies conducted at the Department of Forest Mycology and Pathology, University of Agriculture Science, Uppsala, Sweden, *Pinus sylvestris* seedlings were inoculated in the nursery with various forest mycorrhizal fungi including *Amanita muscaria*, *Lactarius rufus*, *Suillus variegatus*, *Tricholoma albobrunneum*, and an unknown fungus. At the time of outplanting, 10–14% of the root trips were mycorrhizal, consisting of both the inoculated fungi and an indigenous nursery mycorrhizal fungus *Thelephora terrestris*. During the three-year study after transplanting the seedlings in the field, it was observed that the target fungi were replaced by several indigenous forest mycorrhizal fungi which rapidly colonized the remaining uncolonized root system also (Stenstrom and Ek 1990).

In further studies at this University, *Pinus sylvestris* seedlings were inoculated in nursery containers with the assertive mycorrhiza formers *Laccaria laccata* (isolate S-238A, now known as *L. bicolor* isolate), *Hebeloma crustuliniforme* (two isolates), and *Cenococcum geophilum*. At the time of outplanting in the field, 25–90% of the root tips of inoculated seedlings were mycorrhizal with the target fungi, whereas non-inoculated control seedlings were spontaneously colonized by other fungi at rates raging from 25% to 50%. After 1.5 years in the field, the inoculated fungi were still present on the roots, but these were being slowly replaced by indigenous forest species (Stenstrom 1990).

Studies conducted at the Centre for Advanced Studies in Botany, Madras University, Tamil Nadu, India, on *Pinus patula* seedlings showed that the early-stage fungi *Thelephora terrestris*, *Laccaria laccata*, and *Rhizopogon luteolus* occurring in the Nilgiris readily formed ecotmycorrhizae of their respective types on both sterilized and unsterilized soil, whereas the late-stage fungi *Amanita muscaria*, *Scleroderma citrinum*, and *Suillus brevipes* occurring in *Pinus patula* plantations did not establish mycorrhizas in the unsterilized soil (Natarajan and Mohan 1992).

Studies conducted at the Kananaskis Centre for Environmental Research, University of Calgary, Calgary, Canada, on jack pine (*Pinus banksiana*), inoculated with nine mycorrhizal fungi and outplanted on oil sand containment dyke that had been amended with musky peats showed that after one growing season, E. strain, *Hebeloma* spp., *Thelephora terrestris*, and *Laccaria proxima* formed mycorrhizae with 40% of new short roots within 10 cm of the stem. The other introduced fungi (*Cenococcum geophilum*, *Pisolithus tinctorius*, *Astraeus hygrometricus*, *Lactarius paradoxus*, and *Sphaerosporella brunnea*) each formed mycorrhiza with 6% of the short roots on egressed laterals. Of the introduced fungi, only E. strain was present in substantial quantities after three years. The quantity of short roots converted to mycorrhiza by indigenous fungi was 4%, 33%, and 72% after one, two, and three years respectively. At the end of the third year, major indigenous fungi converting short roots to mycorrhizas were E. strain, *Tuber* spp., *Suillus*-like species, *Mycelium radices-atrovirens,* and an unidentified basidiomycete. Compared with uninoculated seedlings inoculation with E. strain and *Thelephora terrestris* resulted in a 2–3-fold increase in shoot weight after two years (Danielson and Visser 1989).

In studies conducted at the Okanagan University College, Department of Biology, Kelowna, Canada, paper birch (*Betula papyrifera*) and Douglas fir (*Pseudotsuga menziesii*) were planted either alone or in mixture and in different proportions and densities on three sites. Field bioassay of 12 seedlings per plot at 4, 16, and 28 months following outplanting showed that *Thelephora*, E. strain, *Rhizopogon* (on Douglas fir only), *Mycelium radicesatrovirens,* and *Cenococcum* mycorrhizae were most abundant. The *Thelephora* mycorrhizae decreased in dominance over the sampling period from 82 to 41% of ectomycorrhiza on birch and from 26% to 15% on Douglas fir. *Rhizopogon* mycorrhizae remained consistently abundant on Douglas fir roots (36% of mycorrhiza at 4 months to 37% at 28 months). By 28 months, 91% of birch and 56% of Douglas fir mycorrhizae were types common to the two species. At 16 and 28 months following outplanting, the evenness of ecotmycorrhizal community on Douglas fir root system was higher in mixed than in single-species plots. Planting density did not affect richness, evenness, or diversity (Jones et al. 1997).

Studies conducted at the INRA, Centre Research Nancy, Equipe Microbial, Forestiere, Champenour, France, showed that *Laccaria bicolor* persisted for at least 10 years after outplanting in a plantation of Douglas fir (*Pseudotsuga menziesii*) inoculated with strain S238 N of *L. bicolor*. The survival of this strain was confirmed by mitochondrial ribosomal DNA analysis. Length polymorphism in fragments of the large subunit of mitochondrial ribosomal DNA (LrDNA) allowed distinction of the haplotypes present in the plantation at the species level. In addition, heteroduplex analysis and sequencing revealed intraspecific polymorphism of LrDNA among the *L. bicolor* sporophores and enabled specific identification of S238 N LrDNA (Selosse, Martin, and LeTacon 1998).

Effect of site in mycorrhizal succession

In studies conducted at the Great Lake Forestry Centre, Forestry Canada, Ontario Region, Canada, black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) seedlings were inoculated in the nursery with five species of ecotmycorrhizal fungi (*Hebeloma cylindrosporum*, *Pisolithus tinctorius*, *Laccaria bicolor*, *L. proxima*, or *Rhizopogon rubescens*) and planted each on two sites (black spruce on peatland and stony loam site and jack pine on stony loam and sandy site). In outplantings, all inoculated fungi except *P. tinctorius* (which did not form mycorrhiza) remained on the root system for two growing seasons, but their presence declined sharply in the second year. *L. bicolor* was the most persistent mycobiont on root system of both these species. Colonization of black source by indigenous mycorrhizal fungi was faster on the stony loam site than on the peat land site. The diversity of wild ectomycorrhizas on the planted seedlings was greater on the peatland and sandy site than on the stony loam site (Browing and Whitney 1992).

Studies conducted at the University of Western Australia, Australia, on outplanted *Eucalyptus globulus* on two sites, namely, gravelly yellow duplex site (site 1) and yellow sandy earth (site 2), showed that inoculant fungi (*Hebeloma westraliense* and *Setchelliogaster* spp.) survived on *E. globulus* roots for a least 12 months. Root colonization by resident fungi increased with time at both sites. At site 2, the increase appeared to be at the expense of colonization by inoculant fungi, which was reduced to less than 10% of fine root length at 12 months. Steaming of soil had little effect on colonization by inoculant ectomycorrhizal fungi at either field site but decreased colonization by resident ectomycorrhizal fungi (Thomson et al. 1996).

Effect of climatic and edaphic factors

Mycorrhizae in field plantings are significantly affected by soil factors such as pH, drainage and moisture, fertility, organic matter, soil temperature and climatic factors such as light and also associated flora such as weeds or associated other plants. Also, for better development of mycorrhiza in the field, seedling lifting, storage, and planting practices should be so designed that maximum number of feeder roots and associated mycorrhizae are retained on the roots (Cordell, Owen, and Marx 1987).

Effect of edaphic factors

Effect of soil moisture

In studies conducted at the Centre for Energy and Environmental Research, San Juan, USA, native mixtures of ectomycorrhizal fungi were found to infect *Populus deltoides* and *Salix nigra* roots primarily in very moist but well-drained soils in both field and controlled experiments (0 to -0.2 MPa), whereas native mixtures of VA-endomycorrhizal fungi infected roots over a much wider range of soil moisture (flooded to -3.4 MPa). Although a moisture gradient experiment showed that endomycorrhizal formation was greater in moist soil than in very dry and flooded soils, this pattern was reversed in field transects along drainage gradients. In the field, infection by VA-endomycorrhizal fungi was the lowest, whereas infection by ectomycorrhizal fungi was high, suggesting possible antagonism among the fungal symbionts. The narrow moisture range for ectomycorrhizal formation and the antagonism between endo- and ectomycorrhizal fungi apparently combine to produce mycorrhizal distribution in nature (Lodge 1989).

In studies conducted at the Lehrstuhl Spezielle Botanik, Universitat Tubingen, Germany, mycorrhizal growth rates were measured monthly

for a year, using a new method in two adjacent plots of natural 60–70-year-old *Picea abies* stand, where one plot was irrigated and the other suffered drought in late summer and autumn. Drought caused higher rate of root dormancy and reduced elongation rate of parent roots, but an increased development of new mycorrhizal last-order laterals, even though fewer growing mycorrhizae were found. Similar results were obtained in a water-stress experiment with pot cultures (Feil, Kottlee, and Oberwinkler 1988).

Studies conducted at the United States Department of Agriculture, Pacific Northwest Research Station, Corvallis, USA, showed that ectomycorrhizal formation strongly influenced seedling survival and growth on sites limited by moisture and temperature. Under such conditions, factors that enhance the rapidity of ecotmycorrhizal formation also produce vigourous seedlings. In periods of adequate moisture, humus supports the highest level of ectomycorrhizae, but during periods of drought, decaying wood becomes the most active site. Following the 1987 fires of southern Oregon and northern California, decaying logs contained 25 times more moisture than soil does and were the center of mycorrhizal activity for pioneering forest vegetation (Amaranthus and Perry 1990).

Studies conducted at the Institute for Mycorrhizal Research and Development, USDA (United States Department of Agriculture), Forest Service, Southeastern Forest Experiment Station, Athens, and Forest Pest Management, USDA, on forestation of routine planting sites and revegetation of acid coal spoils, Kaoline spoils, burrow pits, and severely eroded sites showed that *Pisolithus tinctorius* ectomycorrhizae improve seedling survival and growth on good quality reforestation sites, especially during droughts. On disturbed sites, *P. tinctorius* promotes assimilation and absorption of nutrients, increases water absorption, reduces the absorption of toxic elements, and reduces overall stress inherent on these sites (Marx and Cordell 1998).

Effect of soil temperature

Studies conducted at the Institute of Plant Nutrition, University of Hohenheim, Stuttgart, Germany, on non-mycorrhizal roots of four-year-old Norway spruce and in a stand of 60-year-old Norway spruce showed that uptake rate of both ammonium (NH_4) and nitrate (NO_3) increased with increasing root zone temperature (5–20°C). In the concentration range of 100 to 150 mM, uptake rate of NH_4 was 3–4 times higher than that of NO_3. The preference for NH_4 uptake was also reflected in the minimum concentration values. On supplying ammonium nitrate (NH_4NO_3), the rate of nitrate uptake was very low until the NH_4 concentration had fallen below about 100 mM (Marschner, Haussling, and George 1991).

Studies conducted at the United States Department of Agriculture, Pacific Northwest Research Station, USA, on forest regeneration on disturbed lands showed that ectomycorrhizal formation strongly influenced seedling survival and growth on sites limited by moisture and temperature (Amaranthus and Perry 1990).

Effect of pH

Studies conducted at the Institute of Plant Nutrition, University of Hohenheim, Stuttgart, Germany, on four-year-old non-mycorrhizal Norway spruce and in a stand of 60-year-old Norway spruce showed that the shift from NH_4 to NO_3 uptake was correlated with a corresponding shift from net H^+ ion production to net H^+ ion consumption in the external solutions. The uptake rates of NH_4 were correlated with equimolar net production of H^+. With nitrate nutrition, net consumption of H^+ was approximately twice as high as uptake rates of NO_3. In the forest stand, nitrate concentration in the soil solution was more than 10 times higher than the NH^+ concentration (100 mM), and the rhizosphere pH of non-mycorrhizal roots considerably higher than the bulk soil pH. The rhizosphere pH increase was particularly evident in apical root zones, where the rates of water and nitrate uptake and nitrate reductase activity were also higher (Marschner, Haussling, and George 1991).

Studies conducted at the Department of Biology, Clarkson University, Potsdam, USA, on a forest floor of red pine (*Pinus resinosa*) plantation that was limed showed that differences in numbers of viable ectomycorrhizas between a control and a limed plot were small relative to high amount of spatial and seasonal variability over two field seasons. Numbers of distinct ectomycorrhizal fungal morphotypes were used to calculate diversity indices. Diversity changed seasonally with 2–5 common ectomycorrhizal morphotypes encountered at each date. Liming did not appear to affect diversity of ectomycorrhizal morphological types; however, it did increase the relative frequency of certain ectomycorrhizal fungal morphotypes. The causes for these shifts are not known. Surface phosphatase activities of ectomycorrhizal morphotypes from both limed and unlimed soil were optimal at pH values of 3.5 and 5.0. Seasonal and liming related differences were conserved for phosphatase activity of individual ectomycorrhizal fungal morphotypes, but these differences were minor compared with differences existing among morphotypes. Liming reduced soil phosphatase activity assayed at pH 5.0 and 7.0 in the 012 horizon, but no differences in activity were apparent in the 02 or A1 horizons, although liming altered the pH at these horizons. The decline in activity paralleled changes in soil water content (Antibus and Linkins 1992).

In studies conducted at the Department des Sciences, Forestries, Faculty de Foresterie et de Gevelesia, Universite Laval, Quebec, Canada,

ectomycorrhizal jack pine seedlings produced in growth pouches and inoculated with either *Pisolithus tinctorius* or *Laccaria bicolor* as well as non-inoculated seedlings were exposed weekly to H_2SO_4 solutions adjusted to pH 5.6, 4.0, 3.2, or 2.5 for 14 weeks in plantations. Soil pH was significantly reduced in those plots where the pH was adjusted to 2.5. Seedling growth parameters were not affected by acid treatment. Indigenous colonization was also not affected by acid treatment, but it significantly influenced the persistence of the inoculated mycobionts. *P. tinctorius* was more sensitive than *L. bicolor* to acid treatment. A competition index, used to evaluate post-planting competivity, was higher for *P. tinctorius* in those plants where the watering solution was adjusted to pH 5.6; in all other plots, *L. bicolor* was more competitive with the indigenous mocota (McAfee 1987).

Studies conducted at the Institute of General Botany, Johannes Gutenberg University, Mainz, Germany, on mature Norway spruce (*Picea abies*) forest showed that at the untreated site, mycorrhizal root biomass was lower in the acid humus (pH 3.3) than in the less acid upper (0.5 cm) mineral soil (pH 4.1). During the seven years of soil acidification, the quantity of mycorrhizal roots remained unaffected in the humus and the upper mineral soil, perhaps due to the high buffering capacity of the humus, which prevented a significant alteration of the nutrient status of the roots. However, two years after termination of soil acidification, percentage of mycorrhizal roots in the humus decreased. Six years after liming, there was a two-fold increase in the annual mean quantity of mycorrhizal roots in the humus. Acid irrigation after liming (compensatory liming) had a similar effect on mycorrhizal root production in the humus. However, two years after acid irrigation was terminated, a decrease of mycorrhizal roots in the upper mineral soil (0–5 cm) was observed. As the total amount of mycorrhizal roots in the humus and upper mineral soil remained constant, compensatory liming produced a shift of fine roots to the humus layer. The higher mass of living mycorrhizal roots in the upper mineral soil compared to humus in untreated plot and increased mycorrhizal root mass in humus after liming or compensatory liming are both attributed to increase in pH to 4.5 (Nowotny et al. 1998).

Studies conducted at the Department of Microbial Ecology, University of Lund, Solvegatan, Lund, Sweden, on six-month-old Norway spruce (*Picea abies*) seedlings, planted in a 50-year-old Norway spruce forest in limed (3.8 tons/ha calcium carbonate) or unlimed plots showed that out of six types of mycorrhizas distinguished in these plots, three decreased after liming, two increased, and one was not affected by liming. The effects of total mycorrhizal colonization of roots were opposite for the two years, indicating that the effects of liming are influenced strongly by other environmental factors. Also, in limed plots, the number of saprophytic species producing sporocarps was significantly higher whereas the number of ectomycorrhizal species were lower when compared with control plots (Andersson and Soderstrom 1995).

Studies conducted at the Department of Biology, Clarkson University, Potsdem, USA, on soil cores collected from limed and unlimed plots in a 60-year-old red pine plantation showed that two years of liming caused an increase in pH of the 01 horizon but not of the 012 horizon. Counts of viable mycorrhizae were higher in the unlimed plots on two of the three sample dates. Surface phosphatase activity varied greatly among mycorrhizal morphotypes (Antibus, Linkins, and Melillo 1987).

Effect of site

Studies conducted at the University of Western Australia, Australia, on survival and development of inoculant ectomycorrhizal fungi on roots of outplanted *Eucalyptus globulus* showed that inoculant ectomycorrhizal fungi (*Hebeloma westraliense* and *Setchelliogaster* spp.) survived on roots of *E. globulus* for at least 12 months after outplanting at two sites. The seedlings were outplanted in root bags designed as field containers for young growing trees on two sites, namely, gravelly yellow duplex site (site 1) and yellow sandy earth (site 2). At site 1, colonization of new fine roots by the inoculant fungi was low (less than 20% of fine root length), and inoculation had no effect on growth of *E. globulus*. At site 2, the inoculant ectomycorrhizal fungi colonized up to 30–50% of new fine root lengths during the first six months after planting, and there was corresponding growth response on *E. globulus,* with a close relationship with plant growth at 12 month and root colonization at three months. However, the plant growth at 12 months was related less closely with root colonization at 6 or 12 months. (Thomson et al. 1996)

Studies conducted at the School of Forestry, Fisheries and Wild Life, University of Missouri, Columbia, USA, on black oak (*Quercus velutina*) seedlings inoculated in nursery with *Pisolithus tinctorius* and outplanted at two sites showed that seedling growth and survival differed between two sites. Shading by more abundant herbaceous and low woody vegetation on a comparatively mesic site resulted in greater mortality and less stem growth than on a xeric site during years 1–3. However, seedlings that grew to heights sufficient to overtop neighbouring vegetation on the xeric site exhibited comparatively higher growth rates in years 4–6. As a result, after six years, the xeric site was occupied by a comparatively higher number of small seedlings, whereas the mesic site was characterized by fewer but larger seedlings (Parker et al. 1985).

Effect of climatic factors

Effect of light

In experiments conducted at the Lehrstuhl f. Goebotanik University, Gottingen, Germany, two plots of beech (*Fagus sylvatica*) were selected; one was fertilized once using a high dose of dolomite lime and the other was supplied annually with ammonium sulphate. The effect of these treatments on gas exchange were analyzed with a portable steady-state porometer. On three trees per stand, measurements were taken on the carbon dioxide and water gas exchange rates of leaves under natural conditions as well as under controlled light conditions with an artificial light source to determine the photosynthetic capability. At the same time, continuous measurements of the gas exchange of leaves in the sun-and-shade crown of beech trees were carried out in a beech plot situated in the immediate vicinity of the beech stand, using a field laboratory equipped with temperature-and humidity-controlled cuvettes. A correlation was found between photosynthetic capability and leaf conductance for water vapour. The leaf conductance strongly depended on irradiation. Although cation supply to the limed beech was improved, there was no difference in photosynthetic capability between the beech trees of limed plot and the control plot at the same rate of leaf conductance. Ammonium sulphate fertilizers reduced the photosynthetic capability and hydraulic flow in the root/leaf pathway, as calculated from the daily pattern of evapotranspiration and leaf water potential (Stickan 1989).

In experiments conducted at the School of Forestry, Fisheries and Wild Life, University of Missouri, Columbia, USA, containerized one-year-old seedlings inoculated with *Pisolithus tinctorius* and two-year-old uninoculated bare root seedlings of red oak (*Quercus rubra*) were outplanted in two high quality oak sites, each of which was divided between a shelter wood plot and clear-cut plot. Half of the seedlings were clipped at approximately 8 cm above the root collar. During the first year, after overstorey cuttings, seedlings at the shelter wood plot had significantly lower water potentials, photosynthetic and transpiration rates, and stomatal conductance than did seedlings at the clear-cut plot. The greater level of seedling water stress under the shelter wood plot was associated with restricted root system development and possibly greater competition from overstorey trees for water than the clear-cut plot, where herbaceous vegetation was just becoming established. Following outplanting, containerized seedlings had significantly higher seasonal average midday water potentials than did the bare root seedlings; both had similar photosynthetic and transpiration rates and somatal conductance. Clipping of seedlings did not have any consistent effect on seedling growth or physiological responses during the first year (Crunkilton, Pallardy, and Garrett 1998).

Effect of carbon dioxide

Effect of elevated CO_2 in the atmosphere has been analyzed from various angles. A conceptual model by scientists at Harvard University, Cambridge, USA, is presented on below-ground positive and negative feedbacks affecting plant growth responses to elevated CO_2. The primary negative feedbacks include increased litter C/N ratio, increased immobilization of available nutrients by a large soil microbial pool, and increased storage of nutrients in plants biomass and detritus due to increase in NPP (net primary productivity). Positive feedbacks include increased plant nutrient uptake through alteration in root structure, physiology, or mycorrhizal symbiosis. However, different ecosystems will show different patterns of interacting positive and negative feedbacks within the plant soil system (Bernsten and Bazzaz 1996).

A field experiment conducted at the Institute of Ecology and Resource Management, University of Edinburg, Midlothian, Scotland, on 18 birch (*Betula pendula*) trees grown in open-top chambers in ambient and elevated CO_2 concentrations showed that elevated CO_2 led to a 58% increase in biomass at the end of the experiment. However, elevated CO_2 did not affect relative growth rate during the fourth growing season. Increase in biomass was thus the result of an early effect on relative growth rate in previous years. Trees grown in elevated CO_2 invested more carbon into fine roots and had relatively lesser leaf area than did trees grown in ambient CO_2. In contrast with previous studies, acceleration of growth did not involve a significant decline in nutrient concentrations of any plant tissues. The increased fine roots density may have assisted the trees in meeting their nutrient requirements (Rey and Jarvis 1997).

According to a review published by a scientist at the University NaCl, Cardoba, Conicet, Inst Multidisciplinario Biol Vegetal, Cordoba, Argentina, there is some evidence that plant infection and/or biomass of root symbionts are stimulated by elevated CO_2, but growth enhancement of the host seemingly depends on its degree of dependence on symbiosis and on soil nutrient availability. Experimental and modelling work have suggested the existence of complex feedbacks in the responses of plants and the rhizosphere to CO_2 enrichment. By modifying carbon input from plant to soil, elevated CO_2 may effect the biomass, the infectivity, and the species/isolates composition of root symbionts. This has the potential to alter community structure and ectosystem functioning (Diaz 1996).

In further studies conducted at this university, changes in enzymatic activities, hyphal lengths, and bacterial substrate assimilation were analyzed to tentatively identify the specific components affected

under elevated CO_2 and those which suggest changes in soil organic matter pools. Changes in the functional structures of arbuscular mycorrhizas were also investigated. Most of the microbial variables assessed showed significant and substantial increase under elevated CO_2 of the same order or less than those observed for root mass and length. Total arbuscular and vesicular mycorrhizal infection of roots was higher under elevated CO_2 but the proportion of functional structures was not modified (Dhillion, Roy, and Abrams 1996).

In studies conducted at the University of New Hampshire, Durham, USA, mesocosms of regenerating forest communities in controlled environments maintained at either ambient (375 ppm) or elevated (700 ppm) CO_2 concentrations were established from intact monoliths of organic forest soil and maintained for two years without external inputs of nitrogen. N_{15} pool dilution techniques were used to quantify nitrogen fluxes within the mesocosms at the end of two years. Elevated atmospheric CO_2 concentration increased total plant biomass production, plant C/N ratios, ectomycorrhizal colonization of tree fine roots, changes in tree fine-root architecture and decreased plant NH_4^+ uptake rates, gross NH_4^+ mineralization rates and gross NH_4^+ consumption rates. Also, the proportion of total biomass contributed by white birch (*Beutula papyrifera*) decreased and that contributed by yellow birch (*B. alleghniensis*) increased. Elevated CO_2, however, had no significant effect on the total amount of nitrogen in plant and soil microbial biomass (Berntson and Bazzaz 1998).

In studies conducted at the University of Navada, Department of Environment and Resource Sciences, Reno, USA, one-year-old ponderosa pine (*Pinus ponderosa*) seedlings were planted in undisturbed field soil within open-top chambers, which permitted creation of atmospheres with 700 µL/ litre, 525 µL/litre, or ambient CO_2 concentrations. Soil was amended with ammonium sulphate to increase total nitrogen by 200 µg/g and 100 µg/g respectively, with unamended soil having 900 µg/ g (low nitrogen). Late in the second growing season, a simulated drought episode was imposed by withholding irrigation. Coarse and fine root weights were increased by CO_2 enrichment during the first growing season, being more apparent at 525 µL/ litre CO_2 and high soil N. Shoot/ root ratio decreased with increasing CO_2. After two growing seasons, elevated CO_2 increased seedling diameter, shoot and root volume, and shoot and coarse root weights, most prominently in high N. However, stimulation of seedling growth by 700 µL/litre CO_2 atmosphere exceeded that by 525 µL/litre CO_2 and shoot/root ratio was unaffected by either CO_2 or N. At both harvests, seedlings grown in enriched atmosphere had higher mycorrhizal counts and greater percentages of colonized root lengths but differences among treatments in ectomycorrhizal development were non-significant regardless of quantification method. During the imposed drought episode, xylem water potential of seedlings grown in elevated CO_2 descended below that of seedlings grown in the ambient atmosphere as soil water potential decreased, most notably in predawn measurements (Walker et al. 1998).

Studies conducted at the Harvard University Department Organism and Evolut Biol Labs, Cambridge, USA, on replicate populations of crowded, regenerating stands of *Betula alleghaniensis* grown in ambient and elevated (700 ppm) atmospheric CO_2 concentrations in monoliths of forest soil showed that elevated CO_2 had no significant effects on architectural parameters that improve a plant's ability to forage for and acquire soil resources. In contrast, the intensity and magnitude of mycorrhizal colonization and whole plant C/N ratio were significantly enhanced with elevated CO_2. The allometric scaling relationships between total plant biomass and root biomass was not affected by CO_2, but allometric scaling relationship between root architectural parameters and plant biomass and between fine root biomass and woody root biomass were significantly altered by elevated CO_2. Significant increases in mycorrhizal infection rates and architectural biomass allometers suggest that below-ground competitive interaction within plant populations may be reduced in elevated CO_2 (Berntson, Wayne, and Bazzaz 1997).

Studies conducted at the Smithsonian Tropical Research Institute, Balboa, Panama, on *Beilschmiedia pendula* potted seedlings raised in open-top chambers in a forest clearing and inoculated with VAM fungi showed that VAM inoculation increased the RGR (relative growth rates) at both the ambient and double CO_2 concentrations. RGR was correlated with NAR (net assimilation rate) of the plants. Within the general correlation, in plants with similar RGR, NAR was reduced in VAM plants compared with non-mycorrhizal plants. As RGR is the product of NAR and the LAR (leaf area ratio, which is the ratio of leaf area to plant mass), increases in RGR on VAM plants were the result of increased LAR. Under increased CO_2, the amount of fungus within roots increased in VAM plants compared with those grown under ambient CO_2 and this was associated with a greater post-inoculation depression in leaf growth. Post-inoculation depression in leaf growth and the lower NAR of VAM plants (inplants with similar RGR) indicate that there is increased carbon transfer to soils under increased CO_2 (Lovelock, Kyllo, and Winter 1996).

Effect of organic matter, mulches and associate flora

Studies were conducted at the University of Nevada, Rano, Department of Range, Wild Life and Forestry, USA, to determine the effect of mulching with perforated black plastic in combination with fertilization and induced mycorrhizal symbiosis on

the growth of loblolly pine (*Pinus taeda*) and yellow popular (*Liriodendron tulipifera*) in plantations under intensive short rotation management. Mulching significantly increased height and stem diameter growth of both species, attributable largely to improved water relations resulting from diminished soil surface evaporation and elimination of transpiration losses from competing vegetation. Mulching effects on soil temperature were insufficient to contribute substantially to the growth responses exhibited by mulched trees. Multiple applications of urea nitrogen promoted enhanced growth in both species, an effect accentuated by mulching, but the field performance of trees inoculated in the nursery by selected mycorrhizal fungi was poor relative to that of other treatments investigated (Walker and McLaughlin 1989).

Studies conducted at the United States Department of Agriculture, Pacific Northwest Research Station, Corvallis, USA, on ability of soils to maintain a viable population of ectomycorrhizal fungi following forest disturbance showed that the degree of organic matter lost from a site can influence population of mycorrhizal fungi. Mycorrhizal formation was reduced by 90% and basal area 43% in Douglas fir (*Pseudotsuga menziesii*) seedlings grown in soils where forestry practices drastically reduced organic matter levels. A variety of organic matter types may be critical to support mycorrhizal activity in varying conditions. In periods of adequate moisture, humus supports the highest level of ectomycorrhizae, but during periods of drought, decaying wood becomes the most active site (Amaranthus and Perry 1990). The above study also showed that survival of propagules and input of spores of ectomycorrhizal fungi appear insufficient to maintain mycorrhizal potential of disturbed sites in the absence of living hosts. Certain rapidly pioneering brush species may aid forest regeneration after disturbance by stabilizing population of ectomycorrihal fungi. In southwest Oregon and northern California, species in the families Ericaceae, Fagaceae, Pinaceae, Pyrolaceae, Rosaceae, and Betulaceae form ectomycorrhizae and associate in common with many fungal species. Ericaceaous manzanitas (*Actostaphylos* spp.) and Pacific madrone (*Arbutus menziesii*) rapidly occupy sites following timber harvest and fire. Rapid mycorrhiza formation and improved seedling performance of Douglas fir seedlings were found in association with white leaf manzanita (*Arctostaphylos viscida*) compared to adjacent annual grass areas. In another study, the number and rapidity of ectomycorrhizal formation of Douglas fir seedlings increased with proximity to clumps of green leaf manzanita (*Arctostaphylos patula*). Ectomycorrhizal fungi supported by non-coniferous hosts can actively colonize feeder roots of conifer seedlings. The seedlings benefit by early mycorrhizal formation is critical for establishment on harsh, disturbed sites (Amaranthus and Perry 1990).

Studies were conducted at the Biological Station of the Agricultural University, Kampsweg, The Netherlands, on the effect of removal, doubling, and control treatments of litter and humus layer on ectomycorrhizal root growth in a nitrogen-enriched planted *Pinus sylvestris* stand on podzolic sandy soil and its comparison in an untreated, naturally established *P. sylvestris* on nutrient-poor, non-podzolic sandy soil. Samples collected up to 60 cm depth, six months after manipulation of litter and humus layers showed that root growth and ectomycorrhizal development were greater in the naturally established stand than in all plots of planted stands. The number of ectomycorrhizal root tips in the litter and humus removal plots were generally higher than in control plots in the planted stand. Doubling litter or humus did not significantly affect root length and number of ectomycorrhizal root lips. The N-dissolved, NH_4^+ and NO_3^- concentrations and the organic matter content in the upper 5 cm of mineral soil in the planted stand on podzolic sandy soil were generally higher and the pH significantly lower than in the naturally established stand on non-podzolic sandy soil (Baar 1997).

In studies conducted at the Department of Ecol Evolut and Natural Resources, Rutgers State University, New Brunswick, USA, oak seedlings were planted within and outside patches of trees and shrubs on a closed landfill. Oak root systems were mycorrhizal but root-tip proliferation was improved and ectomycorrhizal composition was influenced by woody debris in the mineral soil. Most surviving oaks were found within patches, but all seedlings showed poor growth, and most tap roots were deflected horizontally above the boundary between surface soil and subsoil soil layers (greater than or equal to 15 cm). Abrupt decrease in pH between surface and subsurface horizons (6.9 vs. 5.3), together with poor drainage and aeration of the latter soil, were probably responsible for poor root growth. Root growth of greenhouse-grown pine and maple seedlings was similarly restricted in pots packed with soil over subsoil material (Parsons, Ehrenfeld, and Handel 1998).

Effect of mycorrhizal fungal mats present on forest floors

Mat-forming mycorrhizal fungi occur in coniferous forests, where they form dense hyphal and/or rhizomorph mats colonizing discrete soil zones. These mats are characterized by increased biological activity and by changes in soil nutrient content and/or availability. In some cases, fungal mats contain greater amounts of microbial biomass than uncolonized soil does. Studies conducted at the Department of Forest Science, Oregon State University, Corvallis, Oregon, USA, on Douglas fir forests have shown that fungal mats have a higher rate of respiration and greater microbial mass than do adjacent soil areas. Fungal mats in these forests also are foci for increased extracellular enzyme

activity and greater rates of acetylene reduction. Nutrient availability is increased in the Douglas fir fungal mats. Increased soil animal populations are present in these mats, possibly as a consequence of enhanced resource availability for these soil biota (Cromack 1990).

Further studies conducted at this university showed that the ectomycorrhizal fungi *Hysterangium setchellii* and *Gautieria monlicola* form extensive hyphal mats with the roots of Douglas fir and other conifers at the surface of the 'A' horizon, often at the interface between mineral soil and litter. Analysis of forest soil with or without ectomycorrhizal mats showed that concentration of dissolved organic matter, oxalate, phosphate, sulphate, hydrogen ion, aluminium, iron, copper, manganese, and zinc were significantly higher in mat than in non-mat soil solutions in both mat types. Significant statistical correlations between dissolved organic matter or oxalate and phosphate indicate that organic acids influence weathering and solubility of phosphate in mat soils. Mean oxalate concentrations were significantly lower in the soil solution from *Hysterangium* mats than in those from *Gautieria*. Organic acids released in rhizosphere by both fungi may provide a local weathering environment that increases availability of phosphate, sulphate, and trace nutrients (Griffiths, Baham, and Caldwell 1994).

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Research findings

Modified wet sieving and decanting technique for enhanced recovery of vesicular–arbuscular mycorrhizal fungi from forest soils

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Introduction

Extraction and isolation of VAM (vesicular–arbuscular mycorrhiza) spores from soil by the wet sieving and decanting technique (Gerdemann and Nicolson 1963) is commonly used in mycorrhizal research. It has been used for a long time, with modifications for practicability and efficiency (Pacioni 1994; Gaur and Adholeya 1994). The standard as well as modified wet sieving and decanting techniques still show difficulties for extraction of VAM spores from soils with higher percentage of sand and silt. The available technique does not provide a clear picture of the spore population, particularly in the loamy sand and sandy soils of humid tropical forests. It is because of this reason that even after repeated washing and decanting, some percentage of spores settle down and remain inside the silt and sand mixture coupled with organic residues. Plant baiting techniques can be deployed to verify the removal of all spores from the sample (Tommerup 1994). This requires more time and labour. At the same time, the viability and specificity of the spores to host plant will affect the results. A slight modification in the available technique will give improved results in extracting and isolating the VAM spores from natural soils. This paper presents the modified wet sieving and decanting technique for more effective extraction and recovery of spores from loamy sand soils in comparison to the standard procedure.

Materials and methods

Twenty-five soil samples were collected using a soil sampler at two depths (0–20 cm and 20–40 cm) from three forest sites (degraded, least degraded, and undegraded) of Arunachal Pradesh, north-east India. A composite mixture was then prepared by mixing all the samples (for each depth and site) in a plastic tray. The VAM spores were isolated using the standard and proposed modified wet sieving and decanting technique in two sets of experiments. Particle size distribution and textural class of the soils were determined by methods of Okalebo, Gathua, and Woomer (1993).

For isolating the VAM spores, approximately 150 ml of tap water was added to 10–20 g of fresh soil in a 250-ml conical flask (glass or plastic). The flask was shaken in a rotary shaker for 10–15 minutes at 100 rpm, and the soil suspension was immediately transferred into a series of sieves (1 mm, 800 µm, 450 µm, 300 µm, 200 µm, 100 µm, and 45 µm). The flask was washed twice or thrice with water into the sieve series. The 1-mm, 800-µm, and 450-µm sieves were removed after completing the washing with a fine jet of water.

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The 300-µm sieve was also washed with a fine jet of water and checked for spores in the residues in the sieve. If spores were present, the residue was transferred into a 250-ml beaker using little water and a fine brush, otherwise it was discarded. The 200-µm, 100-µm, and 45-µm sieves were washed again and the residues transferred carefully in the 250-ml beaker. The water in the beaker was increased up to 150 ml and the beaker shaken with the wrist for 30–60 seconds on the surface of a wooden table and kept standing for 30 seconds. The surface layer containing spores and organic residues was decanted 3–5 times into 50 or 100 ml centrifuge tube with the help of a funnel and little water.

Other steps of centrifugation were same as in the standard technique performed in the other set of experiment. Spore counting was done on Whatman No. 1 filter paper (Gaur and Adholeya 1994) with rectangular grid lines of 0.5 cm^2 drawn on the filter paper which fits exactly one microscopic field under a stereomicroscope (W F 10× eyepiece and 2× or 4× objective).

Results and discussion

Table 1 presents the particle size distribution, profile thickness, and textural class of degraded, least degraded, and undegraded forest soils. Table 2 presents the total number of VAM spores extracted and isolated from the three sites by the standard as well as the modified techniques. On an average, the modified technique gave 21% more recovery of VAM spores over the standard procedure for the three sites at both depths. The possibility of fluctuations in VAM spore distribution due to the heterogeneous nature of soils was eliminated by making a composite mixture of the samples and large number of replicates for the three sites at each depth.

The improved recovery is largely due to the separation of spores from the sedimentary residues that remain unisolated in the standard decanting method. The modification requires 30–60 minutes extra, but the recovery efficiency is greater. Therefore, the proposed modification of wet sieving and decanting technique may be successfully employed for efficient extraction and isolation of VAM spores from loamy sand and other soils with high percentage of sand and silt.

Table 1 Particle size distribution, profile thickness, and textural class of degraded, least degraded, and undegraded forest soils

Parameter	*Degraded*	*Least degraded*	*Undegraded*
Sand (%)	72.8	73.0	74.9
Silt (%)	20.1	18.0	18.1
Clay (%)	7.1	9.0	7.0
Profile (Ah)			
Thickness (cm)	0–2	0–7	0–13
Textural class	Loamy sand	Loamy sand	Loamy sand

Table 2 Total numbers of VAM spores isolated from degraded, least degraded, and undegraded forest soils using standard and modified wet sieving and decanting techniques[a]

		No. of spores/10g of soil	
Study sites	*Soil depth (cm)*	*Standard technique*	*Modified technique*
Degraded	0–20	38	43
	20–40	22	28
Least degraded	0–20	69	81
	20–40	37	51
Undegraded	0–20	109	141
	20–40	57	79

[a] Values presented are means of 25 replicate samples.

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Arbuscular mycorrhizal synthesis in some wetland plants in Kerala

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Introduction

AM (Arbuscular mycorrizal) fungi are ubiquitous in their distribution and occur widely throughout the plant kingdom (Gerdemann 1968). However, little efforts seem to have been directed towards the assessment of AM colonization in wetland plants. Therefore the present investigation has been carried out with an aim to fill in this gap.

Materials and methods

Root samples of 46 wetland plants were collected from various locations of Alappuzha District of Kerala. The physico-chemical characteristics of the soils are shown in Table 1. The roots were cut into 1-cm fragments and stained as per the method described by Phillips and Hayman (1970). For each plant species, three replications were made and from each replication 50 root bits were screened for mycorrhizal infection. The proportion of root infected by AMF was evaluated microscopically by the grid line intersect method (Giovannetti and Mosse 1980).

Table 1 Characteristics of the soils under study

Soil type of locations	*pH*	*Organic carbon (%)*	*Available P (ppm)*	*Soil inundation during the year (months)*
Sandy	5.9	0.54	53.20	5–6
Sandy loam	5.6	0.87	85.13	5–6

Result and discussion

Of the 46 plants screened for mycorrizal colonization, 29 exhibited mycorrhizal association attributed to *Gigaspora* and *Glomus* species in their root system (Table 2). However, there was very less development of vesicles and arbuscules. Among the plants, highest percentage of root colonization and intensity of infection was exhibited by *Impatiens chnensis* (Balsaminaceae), followed by *Lymnophylla gratisima* (Scrophulariaceae) and *Hydrocotyl asiatica* (Umbelliferae). In all other mycorrhizal plants, the percentage root colonization and intensity of infection ranged from 1.1 to 20.7 and 0.3 to 7.5 respectively. Though the plants were grown in aquatic environment, most of them exhibited mycorrhizal infections, indicating that watery environment does not prevent mycorrhiza formation completely, though it induces a spurt in root colonization (Vyas and Srivastava 1988). The root infection by AMF

Table 2 Percentage root colonization and intensity of infection by AMF in wetland plants

Plant species	*Family*	*Mycorrhizal colonization (%)*	*Intensity of infection (IG)*
Acanthospermum hispidum Dc	Asteraceae	6.1	1.5
Acorus calamus L.	Araceae	4.2	0.8
Aeschynomene aspera L.	Fabaceae	4.0	0.7
Alternanthera triandra Lam.	Amaranthaceae	5.9	1.6
Asteracantha longifolia L.	Acanthaceae	0	0
Bergia capensis L	Elatinaceae	5.2	0.5
Blyxa Aubertti Rich	Hydrocharitaceae	0	0
Celosia argentea L	Amaranthaceae	0	0
Colocasia antiquorum Shott	Araceae	17.8	11.3
Commelina beghalensis L	Commelinceae	0	0
Cyanotis axillaris L	Commenlinaceae	16.8	16.8
Cyperus difformis L	Cyperaceae	3.3	0.3
Cyperus Iria L	Cyperaceae	1.9	0.4
Digera muricata (L.) Mart	Amaranthaceae	0	0
Dopatrium junceum Buch	Scrophulariaceae	0	0
Echinochloa colona (L) Link	Poaceae	0	0
Eclipta alba L	Asteraceae	8.9	2.6
Eichhornia crassipes Mart	Pontederiaceae	2.7	1.9
Eriocaulon quinquangulare L	Eriocaulaceae	0	0
Hydrocotyl asiatica L	Umbelliferae	30.8	17.3
Hygrophylla polysperme T. Anders	Acanthaceae	3.3	0.5
Hygrophylla spinosa Heyne	Acanthaceae	2.4	1.2
Impatiens chnensis L	Balaminaceae	43.9	23.7
Ipomoea aquatica Forssk	Convolvulaceae	4.3	0.6
Ipomoea reptans Poir.	Convolvulaceawe	0	0
Ischaemum indicum (Hout.) Merr	Poaceae	0	0
Jussieua repens L	Onagraceae	0	0
Justicia simplex D. Don	Acanthaceae	0	0
Limnanthemum indicum Thw	Gentianaceae	0	0
Limnocarys flava L	Butomaceae	3.7	0.4
Limnophylla gratisima Merr	Scrophulariaceae	33.5	7.4
Limnophylla heterophylla Benth	Scrophulariaceae	3.7	0.4
Lindernia crustaceae L	Scrophulariaceae	2.6	0.5
Ludwigia parviflora L	Onagraceae	18.3	3.5
Monochoria hastata L	Pontederiaceae	2.6	0.6
Monochoria vginalis Persl	Pontederiaceae	0	0
Nymphaea nouchali Burm	Nymphaeaceae	0	0
Nymphaea stellata L	Nymphaeaceae	0	0
Oldenlandia corymbosa L	Rubiaceae	0	0
Oryza sativa var. fatura Sharma & Shastry	Poaceae	4.4	1.4
Pistia stratiotes L	Polygonaceae	20.7	7.5
Polygonum tomentosum Willd	Polygonaceae	11.5	3.8
Vallisneria spiralis L	Hydrocharitaceae	1.1	0.3

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would have probably originated from the floating spores in water or from the spores in the soil during root soil contact during drought period. The mycorrhizal incidence in *Cyperus difformis* and *C. iria* of Cyperaceae further confirms the finding of Gupta and Ali (1993) that the members of family Cyperaceae could be mycorrhizal.

Acknowledgements

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Arbuscular mycorrhizal colonization and incidence of root-knot nematode in some rabi vegetables in eastern Uttar Pradesh: a preliminary survey report

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Introduction

Brinjal (*Solanum melongena* L.), cabbage (*Brassica oleracea* L. var. *capitata*), cauliflower (*Brassica oleracia* L. var. *botrytis*), okra (*Abelmoschus esculentus* L.) Moench), onion (*Allium cepa* L.) potato (*Solanum tuberosum* L.), and tomato (*Lycopersicon esculentum* Mill.) are some important rabi vegetables extensively grown in eastern Uttar Pradesh (UP) and elsewhere. Mycorrhizal association in plants is a rule rather than an exception (Gerdemann 1968). Mycorrhizae help plants in the their mineral nutrition and water absorption. The degree of mycorrhization is indicative of its efficiency, specially when the roots grow in hostile environment (Harley and Smith 1983).

Root-knot nematodes (*Meloidogyne* Goeldi) are important pests in vegetables and cause economic losses in some of them. Eastern UP has remained neglected as no systematic survey has been carried out to ascertain the mycorrhizal association and incidence of root-knot nematodes in this area. This paper reports preliminary data on degree of mycorrhization and root-knot incidence in seven important vegetables in six districts of eastern UP.

Materials and methods

During the survey of six districts of eastern UP, soil and root samples were collected from around brinjal, cabbage, cauliflower, okra, onion, potato, and tomato. Roots were properly washed in running tap water to remove any adhered soil particles and the incidence of root-knot nematode was recorded as measure of gall index on a 0–4 scale (0: no gall formation, 1: 25%, 2: 50%, 3: 75%, and 4: 100% of the root system galled). For assessment of arbuscular mycorrhiza, roots were cleared in near-boiling 10% aqueous solution of KOH for 48 hours. These roots were stained in trypan blue, following several washings in distilled water to drain out KOH (Phillips and Hayman 1970). Stained roots were cut into 1-cm segments, and 85 and 125 such segments were randomly picked up and examined under microscope. The degree of mycorrhization was determined by the Nicolson (1955) formula as follows:

$$\text{Per cent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of segments observed}} \times 100$$

Results and discussion

The degree of mycorrhization in cabbage and cauliflower in various districts ranged from 0 to 5; in brinjal, okra, potato, and tomato from 0 to 80; and

Table 1 Degree of mycorrhization in some important rabi vegetables in six districts of eastern UP

Crop	*Mycorrhizal (%)*	*District*					
		Bahraich	*Faizabad*	*Jaunpur*	*Mirzapur*	*Sultanpur*	*Varanasi*
Brinjal	Range	0–65	0–70	10–70	0–55	0–65	0–55
	Mean ± SD[a]	27 ± 16	41 ± 19	40 ± 20	22 ± 14	20 ± 13	27 ± 15
	n[b]	30	45	25	26	35	40
Cabbage	Range	0–3	0–4	0–2	0–4	0–2	0–4
	Mean ± SD	0.8 ± 0.9	0.7 ± 1	0.7 ± 0.8	0.8 ± 1	0.5 ± 0.8	0.9 ± 1
	n	20	30	20	25	30	25
Cauliflower	Range	0–3	0–5	0–3	0–4	0–2	0–3
	Mean ± SD	0.7 ± 0.9	0.7 ± 1	0.7 ± 0.9	1 ± 1	0.7 ± 0.9	0.7 ± 1
	n	20	30	20	25	30	25
Okra	Range	10–75	5–80	10–65	0–53	0–65	0–65
	Mean ± SD	31 ± 17	29 ± 17	37 ± 18	27 ± 14	24 ± 16	25 ± 17
	n	35	30	15	30	40	30
Onion	Range	25–95	15–95	10–90	20–86	12–80	10–88
	Mean ± SD	68 ± 22	60 ± 22	44 ± 25	56 ± 23	44 ± 17	51 ± 23
	n	25	40	25	20	45	35
Potato	Range	10–70	10–63	10–65	8–57	5–65	0–65
	Mean ± SD	34 ± 18	29 ± 14	31 ± 41	25 ± 13	29 ± 14	26 ± 16
	n	20	35	30	35	30	40
Tomato	Range	16–69	10–65	12–60	0–60	0–65	0-60
	Mean ± SD	37 ± 17	36 ± 18	35 ± 14	27 ± 17	21 ± 16	23 ± 15
	n	30	45	30	40	50	55

[a] Standard deviation; [b] Number of samples

Table 2 Incidence of root-knot nematodes on some cucurbits in six districts of eastern UP

Crop	*Gall index (0–4 Scale)*	*District*					
		Bahraich	*Faizabad*	*Jaunpur*	*Mirzapur*	*Sultanpur*	*Varanasi*
Brinjal	Range	0–4	0–4	0–4	0–4	0–4	0–4
	Mean ± SD[a]	3.1 ± 1.0	3.1 ± 1.1	3.1 ± 11	2.9 ± 1.3	2.9 ± 12	2.9 ± 1.2
	n[b]	30	45	25	26	35	40
Cabbage	Range	0–2	0–2	0–2	0–2	0–2	0–2
	Mean ± SD	0.4 ± 0.7	0.7 ± 0.8	0.5 ± 0.7	0.7 ± 0.8	0.6 ± 0.8	0.5 ± 0.7
	n	20	30	20	25	30	25
Cauliflower	Range	0–2	0–2	0–2	0–2	0–2	0–2
	Mean ± SD	0.4 ± 0.8	0.6 ± 0.8	0.6 ± 0.8	0.6 ± 0.8	0.6 ± 0.7	0.6 ± 0.8
	n	25	30	20	25	30	25
Okra	Range	0–4	0–4	0–4	0–4	0–4	0–4
	Mean ± SD	3.1 ± 1.2	3.1 ± 1.2	2.9 ± 1.3	2.5 ± 1.3	2.7 ± 12	25 ± 1.4
	n	35	30	15	30	40	30
Onion	Range	0–2	0–2	0–2	0–2	0–2	0–2
	Mean ± SD	0.4 ± 0.6	0.5 ± 0.8	0.4 ± 0.7	0.4 ± 0.7	0.5 ± 0.6	0.4 ± 0.6
	n	25	40	25	20	45	35
Potato	Range	0–2	0–3	0–3	0–3	0–2	0–3
	Mean ± SD	0.4 ± 0.7	0.7 ± 0.9	1.0 ± 1.1	0.7 ± 0.9	0.7 ± 0.9	0.8 ± 1.0
	n	20	35	30	35	30	40
Tomato	Range	0–4	0–4	0–4	0–4	0–4	0–4
	Mean ± SD	3.2 ± 1.1	3.1 ± 1.0	3.0 ± 1.2	3.1 ± 1.1	2.8 ± 1.3	2.6 ± 1.4
	n	30	45	30	40	50	55

[a] Standard deviations; [b] Number of samples

in onion from 10 to 95%. The mean value in cabbage and cauliflower varied from 0.5 to 1; in brinjal, okra, potato, and tomato from 21 to 41; and in onion from 44 to 68% (Table 1). It is evident that cabbage and cauliflower, the cruciferous plants, were either non-mycorrhizal in nature or if mycorrhizal then to an extremely low level. Non-mycorrhizal nature of various members of the family Cruciferae was also reported by Smith and Read (1997). Onion, on the other hand, showed the highest degree of mycorrhization compared to the remaining vegetables. Mycorrhizal dependency of this crop has been fully established in the literature (Smith and Read 1997).

Cabbage, cauliflower, and onion appear to be poor hosts of the nematode, as the mean value of the gall index, which is a measure of host preference, did not exceed more than 0.7. Saka and Carter (1987) have categorised them under non-hosts. Potato, which is severely infected by the root-knot nematode at higher altitudes, escaped the infection at many sites. Where infection occurred, it was to a low level, as the average gall index did not exceed 1. Brinjal, okra, and tomato have shown a high degree of susceptibility where they occurred (Table 2). It was also observed, however, that plants showing high degree of mycorrhization failed to show higher numerical rating of gall index, which points towards possible interactions between mycorrhizae and root-knot nematodes under natural conditions (Hussey and Roncadori 1982; Singh, 1997).

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New approaches

Use of Xanthene dyes of visualization of mycorrhizal fungal structures

Xanthene dyes – sulforhodamine G, Phloxine B, rose Bengal, and 4,5,6,7-tetrachloroflorescein – have been used by Melville L, Dickson S, Farquhar M L, Smith S E, and Peterson R L (1998) as fluorochromes for laser scanning confocal microscopy of LR-White resin-embedded mycorrhizae (*Canadian Journal of Botany* 76 (1): 174–178). Sulforhodamine G was the most effective dye, giving an even staining of cell components throughout the material, with minimum background fluorescence of LR-white resin. Confocal microscopy of stained blocks of tissue on a slide, viewed without the use of coverslip, revealed the three-dimensional nature of various mycorrhizal structures. These structures included arbuscules, vesicles and coiled hyphae in arbuscular mycorrhizae, coiled hyphae in orchid mycorrhizae, mantle and Hartig net hyphae in ectomycorrhizae, and intracellular hyphae in arbutoid mycorrhizae. Sections mounted on slides viewed by confocal microscopy provided exceptional clarity of fungal form and cytoplasmic contents and showed the relationship to the plant cells, with negligible background fluorescence. Mounting and staining blocks of resin-embedded material provided a fast and effective technique for the visualization of a variety of plant and fungal tissues. Stain penetration to whole-mounted samples was sufficient to reconstruct these dimensional images, using confocal microscopy.

Identification of Tuber mycorrhizae using multilocus electrophoresis

Multilocus electrophoresis for identification of Tuber ectomycorrhizae has been reported by Urbanelli S, Sallicandro P, De Vito E, Bullini L, Palenzona M, and Ferrara A M (1998) (*Mycologia* 90 (3): 398–395). Commercial value of some species such

as *T. magnatum*, *T. melanosporum*, and *T. aestivum* makes their identification particularly important at the ecotmycorrhizal stage for inoculating practices as well as for tracking the environmental fate of the inoculated species. Electrophoretic ecotmycorrhizal patterns at four enzymatic loci, obtained by modifying methods of enzymatic extraction as well as some staining procedures, allowed the identification of wild and nursery-inoculated ectomyorrhizal samples of Tuber spp. Results obtained indicate that the electrophoretic analysis of gene–enzyme systems is a reliable alternative method to the conventional morphometric criteria of fungal identification. At the same time, it is a useful tool for rapid and simple identification of truffles during the ectomycorrhizal stage for large-scale controlled production.

Centre for Mycorrhizal Culture Collection

Image analyser system: a tool for the characterization of arbuscular mycorrhizal fungi

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The last few years have seen an increased awareness in the study, conservation, and sustainable use of 'biodiversity', a term used to describe variability among living organisms and the ecological complexes of which they are a part. Biodiversity can be exploited in many ways at scientific levels, the most well known and ancient no doubt being in taxonomy and systematics. The other areas include studies of population biology, evolution, and conservation of ecosystems, or for selection of materials for biotechnology.

With the need to explore biodiversity of the world's taxa, international biodiversity initiatives have been taken up. These include political initiatives (Convention on Biological Diversity UNEP, 1992 and Biodiversity Action Plans) as well as scientific initiatives (Global Biodiversity Assessment, Species 2000, Systematics Agenda 2000 International, BioNET, INTERNATIONAL, DIVERSITAS, All Taxa Manuals). Although studies on biodiversity of plants are satisfactory, the situation for fungi is alarming. The working figures for the species numbers in fungi accepted in the GBA (Global Biodiversity Assesment) are 72000 for the known and 1.5 million for the total estimated to occur on earth. Thus, it is necessary that mycologists need to be aware of, and contribute to, such programs. But, this can be done only if researchers come and take up this field seriously. Most researchers regard this field as old-fashioned and outmoded, a relic of a bygone age of science that no longer demands their serious attention. Only a handful of taxonomists are left. Thus, the CMR (Centre for Mycorrhizal Research) has taken up the initiative to promote this basic field of research for mycorrhizal fungi.

Species of AMF (arbuscular mycorrhizal fungi) are currently placed in the Zygomycetes in the order Glomales. In the past two decades, there has been an increase in the number of descriptions of species in the Glomales and the enormous amount of research into the effects some of these fungi have on plant growth. The proliferation of species and the variation in the standards of descriptions inevitably causes much uncertainty in the minds of researchers who want to identify their experimental organisms. Unfortunately, identification of this group of fungi is difficult. AM fungi are obligate symbionts and cannot yet be cultured on artificial media except monoaxenically. Their classification is based on morphology of their resting spores. This implies that, for identification purposes, the fungi must have sporulated and those spores must be separated from the substrate or soil in which they started their reproductive phase on a host plant. There is an urgent need for a system for identifying or at least helping to identify them. Various attempts have been made to devise dichotomous or synoptic keys. The limited use and success of such keys is the result of our limited taxonomic character and materials used for description. Thus, any effort to improve the knowledge of taxonomy of these fungi would be an important step.

The main morphological variables used for the identification of AMF are (a) sporocarp occurrence, shape, colour, and size; (b) peridium occurrence and characteristics; (c) spore colour, size, and shape; (d) spore wall number, colour, thickness,

and ornamentation; and (e) hyphal attachment, shape, and type of occlusions.
The measurement of AMF spores (spore diameter, wall thickness, etc.) is very important for the correct identification of these fungi. At the CMR, the image analyser system is used for this work. Diagnostic slides of the specimen spores are prepared and analysed for several taxonomic characteristics under a compound microscope linked to image analyser.

Image analysis is the science of making geometric and densitometric measurements on images from any source. This system has many advantages over the conventionally used method of micrometer for measurements. The system provides rapid, accurate, and statistically significant data and has an added benefit of acquiring digital images.

The image analyser system comprises a PC having the facility of capturing video images and a software for performing various analyses. The image analyser system Quantimet 500+ is used as a video image analyser, which means that it uses a television camera to sample the image. The camera generates an electronic signal proportional to the intensity of illumination, which is then digitised into picture elements or 'pixels'. At each pixel, the brightness of the image is sampled and it is this digital representation that is analysed by the Quantimet. The system provides several classes of measurements ranging from semi-manual planimetry to semi-automatic particle sizing, including

Figure 1 Image analyser system

- planimetry of length, distance, and area;
- phase percent, area fraction, and volume fraction;
- calibrated densitometry; and
- particle shape and size analysis.

The system is well equipped, having many image processing functions used for image filtering and enhancement for acquiring digital images of the specimen AMF spores. Thus, digital herbaria can also be created with the system.

Recent references

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

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

- *Agroforestry Systems*
- *American Journal of Ecology and Viticulture*
- *Applied and Environmental Microbiology*
- *Applied Soil Ecology*
- *Biological Agriculture & Horticulture*
- *Canadian Journal of Botany - Revue Canadienne de Botanique*
- *Chemosphere*
- *Crop Science*
- *Folia Microbiologica*
- *Hort Science*
- *Journal of Applied Botany - Angewandte Botanik*
- *Journal of Chemical Ecology*
- *Journal of Plant Growth Regulation*
- *Journal of Plant Nutrition*
- *Journal of Plant Nutrition and Soil Science - Zeitschrift Fur Pflanzenernahrung und Bodenkunde*
- *Microbiological Research*
- *Microbios*
- *Mikologiya I Fitopatologiya*
- *Molecular Ecology*
- *Molecular Plant - Microbe Interactions*
- *Mycologia*
- *Mycological Research*
- *Nature*
- *Oecologia*
- *Oikos*
- *Plant Biology*
- *Plant Cell*
- *Plant Science*
- *Planta*
- *Pesquisa Agropecuaria Brasileira*
- *Physiologia Plantarum*
- *Scandinavian Journal of Forest Research*
- *Soil Biology & Biochemistry*
- *Soil Science Society of America Journal*
- *Symbiosis*
- *The New Phytologist*
- *Trees - Structure and Function*
- *Weed Research*

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 corresponding author]
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