Vol. 18 No. 3
October 2006

About TERI

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

Biotechnology and Management of Bioresources Division

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

Mycorrhiza Network

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

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

Mycorrhiza News

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

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Phosphorus mobilization by mycorrhizal fungi. Part II—Factors affecting phosphatase activity

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Effect of phosphorus on phosphatase production by ectomycorrhizal fungi

In studies conducted at the Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA, kinetic constants (Km and Vmax) were determined for surface and extracellular soluble acid phosphatases produced by two ectomycorrhizal fungi (*Cenococcum geophilum* and *Entoloma sericeum*) grown in axenic culture at 2 or 50 µM KH_2PO_4 (potassium dihydrogen phosphate) or sodium inositol hexaphosphate. Results for cultures supplied with inorganic phosphorus were similar to those supplied with organic phosphorus. Surface Vmax estimates were significantly greater for 2 than for 50 µM grown isolates. The presence of constitutive extracellular soluble phosphatase activity resulted in the appearance of inorganic phosphate in media initially supplied with organic phosphorus, suggesting substrate hydrolysis in excess of phosphate uptake. No consistent relationship was found between apparent Km estimates and phosphorus treatments. The two species had surface phosphatase Vmax values differing by as much as two orders of magnitude. The magnitude of the response to phosphorus treatment differed among isolates. The response of phosphatases to changes in phosphorus at concentration comparable with soil solution phosphorus supports the hypothesis that levels of available soil phosphorus may control ectomycorrhizal phosphatase production or activation (Kroehler, Antibus, Linkins 1988).

In studies conducted at the Institute of Terrestrial Ecology, Merlewood Research Station, Grange-over-Sands, Cumbria, UK, acid phosphatase production was determined (by phenol release from disodium phosphate) in non-mycorrhizal and ectomycorrhizal roots of pine (*Pinus contorta*) and birch (*Betula pubescens*) seedlings and also in liquid cultures of six mycorrhizal and two saprophytic fungi (Agaricales). Phosphatase production by mycorrhizal roots was lower than in non-mycorrhizal roots of birch and not significantly different in those of pine (except for mycorrhiza of *Hebeloma crustuliniforme*, where phosphatase production was as in birch). Phosphatase production in birch roots decreased markedly with increasing orthophosphate concentration in solution (but in mycorrhizal roots, increasing again above 60 ppm phosphorus). In pine roots, phosphatase production did not vary with phosphorus concentration. In pure culture, phosphatase production was generally independent of phosphorus concentration. In the presence of sodium inositol phosphate, mycorrhizal fungi generally hydrolysed more phosphorus and released a higher proportion into the filtrate than did saprophytic fungi (*Suillus luteus* behaving more like a saprophyte in this respect). The results suggested that tree roots may exert a negative feedback effect on mycorrhizal phosphatase production; and that the mycorrhizal fungi compete with saprophytic fungi for phosphate in forest litter and release it to the host (Dighton 1983).

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

In studies conducted at the Laboratoire de Recherches sur les Symbioties des Racines, INRA (Institut Nationale de la Recherche Agronomique), Montpellier, France, the total phosphatase activities of a mycelial homogenate and the phosphatase activities in the sediment of high speed centrifugation were measured using sodium phytate or tripolyphosphate in order to see the effect of orthophosphate concentration in the medium on the phosphatase activities of *Suillus granulatus* grown in vitro. These activities were strongly inhibited by the Pi (inorganic phosphorus) in the medium. The activities of tripolyphosphatases seemed to be more inhibited than the activities of phytases at high Pi concentrations. The concentrations at which the activities of tripolyphosphatases in the homogenate and in the sediment, and the concentrations at which the activities of phytases in this sediment were reduced to 50%, were closely related and relatively low (approximately 20 µM Pi). The 50% reduction in activity of total phytases was at a higher concentration than for the others, but in the same range (approximately 70 µM Pi. The activities in the sediment represented a considerable part of the total activities of the mycelial homogenate (50%–90%). These results illustrate the importance of the extracellular activities of the mycelia (Bousquet, Mousain, Salsac 1986).

In studies conducted at the Department of Plant Pathology, University of Wisconsin, Madison, Wisconsin, USA, the ectomycorrhizal fungus, *Hebeloma arenosa*, was assayed for surface-accessible acid phosphatase activity *in vitro* on roots of red pine (*Pinus resinosa)* seedlings. *H. arenosa* was grown in defined liquid media containing 0, 17, 34, 68, or 136 mg of phosphorus per litre for four weeks. When assayed for acid phosphatase activity with p-nitrophenyl phosphate, 7.3 µM of orthophosphate were released per gram dry weight of fungal tissue. There was no effect of added phosphorus on enzyme activity in the cultures. Red pine seedlings were grown in sparta loamy fine sand amended with 0, 17, 34, 68, or 136 mg phosphorus per kilogram as superphosphate, with and without *H. arenosa* inoculum. Mycorrhizal roots had greater enzyme activity than non-mycorrhizal roots of seedlings grown in soil with similar phosphorus amendments. This was determined by assays of orthophosphate release from two salts of inosital hexaphosphate (sodium and potassium magnesium) and from p-nitrophenyl phosphate. The greater acid phosphatase activity by roots mycorrhizal with *H. arenosa* may be one mechanism for improved phosphorus nutrition through the formation of a pool of phosphorus released from sources unavailable for direct intake (MacFall, Slack, Iyer 1991).

Studies conducted at the Department de Biologia Geral, Universidade Federal de Viçosa, Minas Gerais, Brazil, showed that the acid phosphatase activity of five ectomycorrhizal fungi increased with decreasing phosphorus levels in the culture medium, but this activity decreased with the decrease of phosphorus in the culture medium in respect of *Rhizopogon nigrescens*. The highest phosphatase activity was recorded for *Paxillus involutus*. The P0/P128 (phosphatase/inorganic phosphorus) ratio ranged from 1.5 (*R. nigrescens*) to 110 in *P. involutus*. In studies on *Pisolithus tinctorius* 185, mycelial dry weight yield and phosphorus and calcium content, but not nitrogen content, increased with an increase in phosphorus level. Magnesium content was lower when phosphorus was present but did not change with increasing phosphorus in the culture medium. There was a high correlation between magnesium and phosphatase activity, suggesting an involvement of protein synthesis on enzyme reduction by phosphorus starvation (Pacheco, Cambraia, Kasuya 1991).

Studies conducted at the Katholieke University Leuven, Dev Biology Laboratory, Kardinaal Mercierlaan, Louvain, Belgium, showed that the external mycelia of *Thelephora terrestris* and *Suillus luteus*, associated with *Pinus sylvestris* roots, exhibited a substantial extracellular acid phosphatase activity. The activity was positively correlated with the ergosterol concentration in the growth substratum and decreased with increasing phosphorus nutrition. The pioneer species, *T. terrestris*, grew best at a high Pi nutrition level whereas *S. luteus*, a 'late-stage' mycobiont, produced more active biomass at a low Pi nutrition level. Also, the phytase activity of the external mycelia could not be detected; although, at the root surface, phytase activity was observed. Mycorrhizae had significantly higher activities than uninfected roots. The addition of a relatively high concentration of a soluble phytate to the growth substratum resulted in an increased RGR (relative growth rate) in both mycorrhizal and non-mycorrhizal plants. The influence of the mycorrhizal fungi on the use of the phytate-phosphorus was small, despite the phytase activity of the mycorrhizal feeder roots (Colpaert, VanLaere, VanTichelen, *et al.* 1997).

Effect of phosphorus on phosphatase production by vesicular arbuscular mycorrhizal fungi

In greenhouse pot experiments conducted at the Universidad de la Frontera, Tamuco, Chile, seedlings of wheat cultivars, Dalcahue, Malihue, Carahue, and Naofen were grown in the typic low phosphorus volcania soil of South Chile, with and without 300 kg of phosphorus pentaoxide per hectare and harvested at 21, 42, 63, 84, and 96 days after planting. VAM (vesicular arbuscular mycorrhiza) infection was similar among all

genotypes. VAM root colonization was increased up to 63 days before decreasing up to 84 days after planting. Root surface acid phosphatase activity did not differ significantly among cultivars, and the enzyme was significantly influenced by phosphorus. When plants were grown without phosphorus fertilizer, maximum enzyme activity was reached at 63 days for Carahue and Dakahue and 43 days for Naofen and Malihue. Phosphatase activity (microg P-nitrophenol released per gram of dry root) was highest at 21 days but quickly declined in the later samplings. When phosphorus was applied, total infected root length increased up to 63 days. Naofen and Malihue presented the greatest amount of exuded enzyme, which increased soil enzyme content by 12% and 20%, respectively (Rubio, Moraga, Borie 1990).

In studies conducted at the Faculty of Horticulture, Chiba University, Matsudo 271, Japan, on phosphatase specific to infection of marigold (*Tagetes patula* cv. *Bonanza*) roots by the VAM fungus, *Glomus etunicatum* , the infection-specific phosphatase (ISPase) was detected in the mycorrhizal root extract from roots of 2–10 week old plants from the beginning of the infection (4 week old plant) by an electrophoretical technique. The activity increased as the infection rate increased, and decreased at the stationary phase of host growth when the infection rate was still high. Studies on the effect of phosphorus fertilization on ISPase activity and mycorrhizal growth promotion in 4–8 week old plants showed that mycorrhizal plants without phosphorus fertilization showed a greater increase in shoot fresh weight and higher ISPase activity than the plants with phosphorus fertilizer. The ISPase was partially purified by ammonium sulphate, hydrophobic chromatography, and gel filtrartion, and characterized. The optimum pH was 7.5, 1 µM phosphate ion inhibited half of the activity. The enzyme hydrolysed inosine 5'-diphosphate effectively and polyphosphate moderately. The possibility of using ISPase as an indicator for the growth response of mycorrhizal plants and the metabolic role of this enzyme were discussed (Ezawa and Yoshida 1994).

In studies conducted at the Department de Fisiologia Aegetal, Instituto de Investigaciones Agrobiologicas de Galicia, CSIC, Apartado 122, Spain, phosphatase activity was studied in three acid steam-sterilized soils, in which mycorrhizal and non-mycorrhizal red clover plants had been grown for 5.5 months at different Ca $(H_2PO_4)_2$ H_2O (calcium dihydrogen phosphate) doses (0, 25, 50, 100, and 200 ppm). The same treatments were established in two other sterilized and unsterilized soils. High acid phosphatase activity could be observed in all three soils, while alkaline phosphatase activity had no significance. The other two soils showed higher acid phosphatase activity in the sterilized treatments. At low phosphorus doses (0–50 ppm) in the sterilized soil, VAM symbiosis effective to enhance plant growth determined less acid phosphatase activity in soil (Sainz, Trasar, Arines, *et al.* 1987).

In studies conducted at the Department of Botany and Plant Pathology, Michigan State University, E Lansing, USA, maize (*Zea mays* cv. Great Lakes 586) plants were grown under five different levels of soil phosphorus, either in the presence or absence of formononetin or the VAM fungus, *Glomus intraradices*. Physiological differences in mycorrhizae were detected very early in the development of symbiosis, before the onset of nutrient-dependent responses. Under low phosphorus levels, VAM roots accumulated a greater shoot dry weight (13%), root phosphorus concentration (15%), and protein concentration (30%) than non-mycorrhizal roots, although root growth was not statistically significantly different. At higher phosphorus levels, mycorrhizal roots weighed less than non-VAM roots (10%) without a concomitant host alteration of growth or root phosphorus concentration. Mycorrhizal colonization decreased as soil phosphorus increased. Formononetin treatment enhanced colonization of the root by *G. intraradices* and partially overcame inhibition of VAM colonization by high soil phosphorus concentrations. ACP (acid phosphatase) and ALP (alkaline phosphatase) activities were closely related to the level of fungal colonization in maize roots. ACP activity in maize roots responded more to soil phosphorus availability than did ALP activity (38% more). These results suggest that ACP was involved in the increased uptake of phosphorus from the soil, while ALP may be linked to active phosphate assimilation or transport in mycorrhizal roots. Thus, soil phosphorus directly affected a number of enzymes essential in host–endophyte interplay, while formononetin enhanced fungal colonization (Fries, Pacovsky, Safir, *et al.* 1998).

Studies conducted at the Mycology and Plant Pathology Laboratory, Department of Botany, Osmania University, Hyderabad, India, on seedlings of *Terminalia arjuna*, grown in polythene bags in sterilized soil treated with *Glomus mosseae*, *G. fasciculatum*, and rock phosphate separately, or in various combinations showed that acid phosphatase activity increased to a maximum in *G. fasciculatum* roots followed by *G. mosseae* + phosphorus and *G. mosseae* + *G. fasciculatum* treated roots. The acid phosphatase activity in shoots was maximum in *G. mosseae* + phosphorus treated plants. All other combinations had reduced acid phosphatase activity. Alkaline phosphatase activity was considerably lower than the acid phosphatase activity in the roots and shoots of all the VAM fungi treated Terminalia plants. Alkaline phosphatase activity was maximum in roots of *G. fasciculatum* + phosphorus and in shoots of *G. mosseae* + phosphorus, followed by phosphorus

treated plants. Positive correlation was noted between acid phosphatase activity and phosphorus concentration (Bhadraiah, Kankadurga, Ramarao, *et al.* 1999).

Effect of nitrogen fertilization on phosphatase production by mycorrhizal fungi

In studies conducted at the Institute of Dendrology, Polish Academy of Science, Kornik, Poland, cell wall-associated acid phosphatase activity was analysed in Scots pine roots treated with urea, ammonium tartrate, and calcium nitrate supplied in nutrient solutions at different levels. The Ca:Mg ratio in urea and ammonium tartrate as nitrogen source was 1:3, but with calcium nitrate, the Ca:Mg ratios were 2.7:3, 9.5:3, and 17.9:3 for solutions of 10, 50, and 100 ppm nitrogen, respectively. One-year-old seedlings with unidentified ectomycorrhizae were transferred from a nursery into pots containing forest soil and fertilized twice a week over a 10 month period. The highest activity of acid phosphatase was found in pine roots supplied with ammonium tartrate and was associated with the highest growth of pine roots. The lowest enzyme activity was found in roots of seedlings treated with calcium nitrate. It is suggested that the Ca:Mg ratio can influence acid phosphatase activity in the pine roots (Kieliszewska-Rokicka 1990).

Effect of different factors on phosphatase activity

Effect of pH on phosphatase activity of mycorrhizal fungi

Studies conducted at the Department of Biology, Clarkson University, Potsdam, New York, USA, on isolates of the ectomycorrhizal fungi, *Cenococcum geophilum*, *Hebeloma pusillum*, and *Entoloma sericeum*, grown in axenic culture to study the effects of assay pH on the activity of surface acid phosphatases showed that four of the six isolates examined demonstrated distinct pH optima at pH 5.0; one isolate showed optimal activity at pH 4.5. None of the fungi examined produced significant surface alkaline phosphatase activity. The above findings demonstrated the importance of considering assay pH when making comparisons on interspecific and intraspecific differences in acid phosphatase activity (Antibus, Kroehler, Linkins 1986).

Studies were conducted at the UFR de Science du Sol, INRA-ENSA, 2 place Pierre Viala, Montpellier, Cedex, France, on the effect of pH and ionic strength on the interaction with montmorillonate of two acid phosphatases secreted by ectomycorrhizal fungus, *Hebeloma cylindrosporum*. The interaction of extracellular enzymes with the solid phase of the soil affects their mobility and their catalytic properties. In particular, absorption on clay minerals moves the optimum pH of the catalytic activity towards alkaline values. Two conflicting interpretations of this phenomenon have been proposed—that is, a surface pH effect and a pH dependent modification of the conformation of the absorbed enzymes. Studies were conducted at the above laboratory to assess the two mechanisms by adsorption on montmorillonite of two extra cellular acid phosphatases of *H. cylindrosporum* and its consequences on catalytic activities. The results were better interpreted by a pH dependent modification of enzyme conformation due mainly to electrostatic interactions with the clay surface. At low pH, both the enzymes and the clay are negatively charged, and adsorption decreases. Adsorption and modification of conformation are largely irreversible, which should be taken into account when considering the fate of enzymes in soil. Finally, the comparison with the effect of clays on the catalytic activities of intracellular enzymes suggests a selection pressure of the soil solid phase leading to more stable extracellular enzymes (Leprince and Quiquampoix 1996).

Studies conducted at the CNRS, Centre de Pedologie Biologique, Vandoeuvre-les-Nancy, France, on extraradical hyphae of *Glomus intraradices* or *G. claroideum* extracted from root-free sand of two-compartment pot cultures and used to determine fungal phosphatase activity (P-nitrophenyl phosphate hydrolysis) showed that there was an overall maximum enzyme activity at pH 5.2–5.6 for both fungi, with a possible secondary maximum at pH greater than or equal to 8.8 for *G. claroideum*. Separation of the phosphatase activity into external soluble, wall-bound, and internal fractions revealed that up to 70% of the measured activity was associated with the hyphal wall, and the rest with internal structures. Exuded phosphatases were not found in measurable amounts (Joner and Johansen 2000).

Effect of temperature and/or season on phosphatase activity

In studies conducted at the Department of Biology, Clarkson University, Potsdam, New York, USA, isolates of ectomycorrhizal fungi, *Cenococcum geophilum*, *Hebeloma pusillum*, and *Entoloma sericeum*, were grown in axenic culture at 12 °C and 20 °C to determine whether the Arrhenium activation energies of surface phosphatases were affected by temperature acclimation. Arrhenius activation energy values were not affected by lowering the growth temperature, but six isolates examined demonstrated distinct pH optima (Antibus, and Linkins 1986).

Studies conducted at the Department of Botany, School of Life Sciences, North-Eastern Hill

University, Shillong, India, on seasonal fluctuation in dehydrogenase, phosphatase, and urease activity in the ectomycorrhizal region in different age groups of *Pinus kesiya* showed an increase in enzyme activities with an increase in the age of the pine. Marked seasonality in enzyme activity was observed. Minimum enzyme activity was noted during the winter and maximum during autumn, summer, and spring for phosphatase, dehydrogenase, and urease, respectively. Except phosphatase activity, available phosphorus nutrient status of soil and soil moisture did not show any correlation with enzyme activity. In earlier studies at the same university, however, it was observed that soil temperature and moisture influenced the dehydrogenae and phosphatase activity in the ectomycorrhizal region of Pinus kesiya (Sharma and Rao 1987; 1988).

In studies conducted at the Department of Biology, University of Leeds, Leeds, West Yorkshire, England, Hebeloma strains of arctic and temperature origin, grown at 22 °C or 6 °C, were assayed for wall-bound and extracellular acid phosphomonoesterase across a temperature range of 2–37 °C. A cold active extracellular acid phosphomonoesterase was induced in all the arctic strains and most of the temperature strains tested only when grown at 6 °C. Such enzymes are suggested to be an adaptation to low soil temperatures and are discussed in the context of ectomycorrhizal access to soil phosphatemonoesters at a low temperature (Tibbett, Grantham, Sanders, *et al.* 1998).

Studies conducted at the Centre de Pedologie Biologique, Vandoeuvre, Nancy, France, on extraradical hyphae of *Glomus intraradices* or *G. claroideum* extracted from root-free sand of two-compartment pot cultures showed that phosphatase activity at pH 5.2 decreased sharply with temperature, with 4.5% and 10.5% of the enzyme activity in *G. Intraradices and G. claroideum*, respectively, remaining at 5 °C relative to that at 37 °C (Joner and Johansen 2000).

Effect of soil moisture on phosphatase activity

Studies conducted at the Department of Botany, School of Life Science, North-Eastern Hill University, Shillong, India, on VAM in rhizosphere of paddy grown in dryland (*jhum*) or wetland showed that moisture content and organic carbon of soil had a positive correlation with phosphatase and dehydrogenase activities, and a negative correlation with mycorrhizal infection and rate of nitrification (Jyrwa and Sharma 1988).

In studies conducted at the USDA (United States Department of Agriculture), Forest Service, Pacific Northwest Research Station, Forest Science Laboratory, Corvallis, USA, 17 isolates of ectomycorrhizal fungi, encompassing five genera and eight species were compared for acid phosphatase, alkaline phosphatase, and nitrate reductase activity on mesic and xeric sites. Generally, the Douglas-fir (*Pseudotsuga menziesii*) associates, which dominate on mesic sites, had higher acid phosphatase activity than pine (*Pinus*) associates, which mostly occupy xeric sites. However, pine associates from mesic sites also have higher acid phosphatase activity (for example, *Suillus tomentosus*). In four isolates of *Amanita muscaria*, the effect of the site was also apparent. Two of them, which had higher acid phosphatase activity, were isolated from mesic sites (Ho 1989).

Studies conducted at the Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA, on surface phosphatase activity of arctic willow ectomycorrhizal roots on natural moisture gradient showed that site differences in enzyme activity were not significant, although phosphatase activities among the mantle colour types assayed were significantly different. Sites assayed were different in slope angle, drainage, soil composition, organic soil depth, and vegetation cover. However, site differences in colour types infecting roots were significant. It appears that the extent of infection by various fungal types is more important in determining total phosphatase activity than were changes in the enzyme activity of any one colour type (Kroehler and Linkins 1997).

Effect of host specificity on phosphatase activity

Studies conducted at the USDA, Forest Service, Northwest Research Station, Corvallies, USA, on acid phosphatase, alkaline phosphatase, and nitrate reductase activity of selected ectomycorrhizal fungi showed that the isozyme pattern of the genus *Suillus* appeared to be separated by host groups. Other isolates from only one species were also different more or less by the host groups. They shared at least one band within host groups, except for the two isolates of *Paxillus involutus* from different hosts. Thus, the host may have affected phosphatase enzyme activity (Ho 1989).

In further studies at the above research station, 64 pure culture isolates, belonging to 27 species in the genus Rhizopogon (hypogeous Basidiomycotina) were analysed for acid phosphatase allozyme patterns and acid phosphatase, alkaline phosphatase, and nitrate reductase activity. The results showed strong relationships in Rhizopogon to either host associates or site conditions. Significant differences in enzyme activity were evident among and within species. With cluster analysis, acid phosphatase allozyme patterns revealed isolate groups based on host specificity and geographic location. Such grouping indicates that acid phosphatase allozyme patterns are related to the ecological provinces of host tree species. Genetic variation in acid phosphatase activity may be

maintained by geographic separation and enable evolution of edaphic ecotypes within Rhizopogon species (Ho, Molina, Castellano, *et al.* 1990).

Effect of pollutants on phosphatase activity

Studies were conducted at the Department of Pedology and Geology, University of Agriculture, Lemedelska, Czechoslovakia, to determine acid phosphomonoesterase activity of ectomyrorrhizal roots of Norway spruce pure stands exposed to pollution. The release of orthophosphate ions from organic compounds is essential for continuous phosphorus cycling in forest ecosystems. An important stage of this process in coniferous forests of the temperate zone is the production of acid phosphomonoesterase by ectomycorrhizal fungi. The effect of artificial and natural pollutant inputs during repeated short periods of high concentration on the activity of the specific enzyme thus needs to be studied. The monitoring of the seasonal dynamics of the activity of acid phosphomonoesterase from February 1989 to January 1990 showed that there was a significant decrease of acid phosphomonoesterase activity in ectomycorrhizal spruce roots as affected by pollutant input. The amount of acid phosphomonoesterase activity may thus become one of the characteristics of the changing biochemical processes in soils under the effects of air pollution (Rejsek 1991).

In studies conducted at the Institute of Botany of the Jagiellonian University, Lubier 46, Cracow, Poland, the influence of cadmium dust (containing cadmium, lead, copper, zinc, silicium, and other elements) on acid phosphatase activity of *Pisolithus arrhizus* was observed by means of electron microscopy. Dust-treated mycelium showed increased activity of the enzyme, especially on the surface of the cell wall. There was an increase in autophagic vacuoles marked by a strong phosphatase reaction. An increase in the number of hyphae with diffused enzyme activity within the cytoplasm coincided with a decrease of life-span of the fungus, rapid changes in the microplasm stage, earlier closing of the dolipores, and presumably the earlier autolysis of cell cytoplasm. Hyphae showing strong autolytic activity were separated from other hyphae by the material deposited within the doliporus and this whole area was devoid at that stage of acid phosphatase activity (Turnau and Dexheimer 1995).

In studies conducted at the Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Madras, India, *Laccaria laccata* was cultured on Palmer and Hacskaylo medium amended with 0.15, 0.30, 0.45, and 0.60 µM copper or nickel per litre as $CuCl_2$ or $NiCl_2$. *L. laccata* was more tolerant to copper and nickel. Increasing copper and nickel concentrations induced the increase of acid phosphatase activity (maximum at 0.15 µM) in *L. laccata* (Periasamy and Raman 1995).

Effect of organic matter on phosphorus mobilization by mycorrhizal fungi

Studies conducted at the Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK, on the relationship between colonization of FHOM (fermentation horizon organic matter) by an ectomycorrhizal fungus and the activities of enzymes involved in key processes of phosphorus mobilization showed that the activity of phosphomonoesterase increased in FHOM that had been colonized for 28–50 days. The amounts of this enzyme in FHOM colonized for 50–98 days, however, decreased to below that found in uncolonized material (Gray and Read 1995).

In studies conducted at the Department of Biosciences, Division of General Microbiology, University of Helsinki, Helsinki, Finland, on Scots pine seedlings colonized by *Suillus bovinus* or *Paxillus involutus* and grown on humus in two-dimensional Perspex microcosms, soluble proteins from uncolonized short roots, whole mycorrhizal root tips, or dissected mantle and core fractions, fungal strands, and outer-most soil colonizing fine hyphae were subjected to native polyacrylamide gel electrophoresis and stained to detect different enzymes. High fungal acid phosphatase activities were detected in mycorrhizae and fine hyphae of *S. bovinus*, supporting findings of active phosphatase activity at the fungal interface in the Hartig net region and in the fine hyphal margins of extra matrical mycelium actively colonizing the humus. All compartments in the *P. Involutus* mycorrhizosphere had weaker acid-phosphatase activities (Timonen and Sen 1998).

Mobilization of intracellular hyphal phosphorus by mycorrhizal fungi

In studies conducted at the Laboratoire de Recherches sur les Symbiotes des Racines, INRA, Cedex, France, acid phosphatase activities in the soluble fraction of *Pisolithus tinctorius* homogenates, cultured on media without or with 100 µM of inorganic phosphorus, were assayed using sodium polyphosphate. Phosphatase activities increased with decreasing lengths of polyphosphate chains. The observations suggested that the accumulations of inorganic polyphosphates in *P. tinctorius* mycelia grown on inorganic phosphorus rich media may be hydrolysed by intracellular phosphatases when the phosphorus supply became limited, a conclusion corroborated by 31P NMR (nuclear magnetic resonance) spectroscopy (Tillard, Bousquet, Mousain, *et al.* 1990).

In studies conducted at the Instituto Coltivazioni Arboree dell'Universita, V. Giuria, Torino, Italy, a biochemical approach was used to

study polyphosphate accumulation when mycorrhizal leek plants grown in a soil lacking phosphorus were subjected to phosphorus fertilization. Polyphosphate, which is a common storage form of phosphorus in fungi, was extracted in hot water, purified on active charcoal, and measured by the colorimetric reaction with toluidine blue or by the release of phosphorus after hydrolysis for seven minutes in hot 2M hydrochloric acid. Acid and alkaline phosphatases and exopolyphosphatase activities were measured with photometric methods while endopolyphosphatase activity was assessed by viscosimetry. Extraradical hyphae contained about 2 nMol polyphosphate per milligram fresh weight, as assessed by toluidine staining and hydrolysis. After soil phosphorus fertilization (2 µM potassium dihydrogen phosphate), the polyphosphate concentration reached in two hours, a maximum of 4 nMol per milligram fresh weight, then it slowly decreased. Following phosphorus fertilization, the activities of acid and alkaline phosphatases in extraradical hyphae decreased. No exopolyphosphatase activity was found in the extraradical mycelium while endopolyphosphatase activity was present. In mycorrhizal roots, polyphosphate was assessed by hydrolysis. Polyphosphate was present at 3 nMol per 50 milligrams fresh weight and after phosphorus fertilization, it increased to 10 nMol per 50 milligrams fresh weight. Maximum concentration was observed about 6 hrs from the time of soil phosphorus fertilization, thus suggesting that part of the polyphosphate which is accumulated in extraradical mycelium may be relocated to intraradical hyphae (Schubert and Wiemken 1990).

References

Antibus R K and Linkins A E. 1986
Cyanide-resistant respiration in *Cenococcum geophilum*
Transactions of the British Mycological Society **86**(1): 163–165

Antibus R K, Kroehler C J, and Linkins A E. 1986
The effects of external pH temperature and substrate concentration on acid phosphatase activity of ectomycorrhizal fungi
Canadian Journal of Botany **64**: 2383–2387

Bhadraiah B, Kankadurga V V, Ramarao P, Manoharachary C. 1999
Effect of VAM fungi and rock phosphate on phosphatase activities in *Terminalia arjuna*
National Conference on Mycorrhiza. Section 3 (Poster): Physiology and Biochemistry. 5–7 March 1999

Bousquet N, Mousain D, and Salsac L.1986
Influence de Orthophosphate sur les activities phosphatases de *Suilllus granulatus* en culture in vitro
Physiol Veg. **24**(2): 153–162

Colpaert J V, VanLaere A, VanTichelen K K, VanAssche J A. 1997
The use of inositol hexaphosphate as a phosphorus source by mycorrhizal and non-mycorrhizal Scots pine (*Pinus sylvestris*)
Functional Ecology **11**(4): 407–415

Dighton J. 1983
Phosphatase production by mycorrhizal fungi
In *Tree root systems and their mycorrhizas* (edited by Atkinson D et al). The Hague, Netherlands 1983. 455–462

Ezawa T and Yoshida T. 1994
Characterization of phosphatase in marigold roots infected with vesicular-arbuscular mycorrhizal fungi
Soil Science and Plant Nutrition **40**(2): 255–264

Fries L L M, Pacovsky R S, Safir G R, Kaminski J. 1998
Phosphorus effect on phosphatase activity in endomycorrhizal maize
Physiologia Plantarum **103**(2): 162–171

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

Ho I. 1989
Acid phosphatase, alkaline phosphatase, and nitrate reductase activity of selected ectomycorrhizal fungi
Canadian Journal of Botany. **67**(3): 750–753

Ho I, Molina R, Castellano M A, Luoma D. 1990
Enzyme activity and allozyme patterns in the genus Rhizopogon
Innovation and Hierarchical Integration. Proceedings of the eighth North American Conference on Mycorrhiza. 5–8 September, 1990. p. 142.

Joner E J and Johansen A. 2000
Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi
Mycological Research **104**: 81–86

Jyrwa S and Sharma G D. 1988
VAM, phosphatase, dehydrogenase and nitrification in root region of paddy under different agricultural practices
Mycorrhizae for Green Asia: Proceedings of the first Asian Conference on Mycorrhizae. pp. 142–144

Kieliszewska-Rokicka B. 1990
Acid phosphatase activity in roots of Scots pine (*Pinus sylvestris* l) seedlings fertilized with different nitrogen sources
Agriculture, Ecosystems and environment **17**(1–4): 229–234

Kroehler C J, Antibus R K, and Linkins, A E. 1988
The effects of organic and phosphorus concentration on the acid phosphatase activity of ectomycorrhizal fungi
Canadian Journal of Botany **66**(4): 750–756

Kroehler C J and Linkins A E. 1997
Surface phosphatase activity of arctic willow ectomycorrhizal roots on a natural moisture gradient
In: Proceedings of the Seventh North American Conference on Mycorrhiza, 3–8 May 1987, Gainesville, Florida.

Leprince F and Quiquampoix H. 1996
Extracellular enzyme activity in soil: effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus, *Hebeloma cylindrosporum*.
European Journal of Soil Science. **47**(4): 511–522

MacFall J, Slack S A, and Iyer J. 1991
Effects of *Hebeloma arenosa* and phosphorus fertility on root acid phosphatase activity of red pine (*Pinus resinosa*) seedlings.
Canadian Journal of Botany **69**(2): 380–383

Pacheco S, Cambraia J, and Kasuya M C M. 1991
Effect of different levels of phosphorus on acid phosphatase activity and mineral composition of some ectomycorrhizal fungi
Revista de Microbiologia. 22(4): 345–348

Periasamy K and Raman N. 1995
Effects of Cu and Ni on acid phosphatase activity of an ectomycorrhizal fungus, *Laccaria laccata*
Indian Journal of Experimental Biology **33**(6): 537–538

Sharma G D, and Rao C S. 1988
Seasonal variation in enzyme activities in the ectomycorrhizal region of *Pinus kesiya*.
Mycorrhizae for Green Asia : Proceedings of the first Asian Conference on Mycorrhizae. pp. 159–161.

Rejsek K. 1991
Acid phosphomonoesterase activity of ectomycorrhizal roots in Norway spruce pure stands exposed to pollution
Soil Biology Biochemistry **23**(7): 667–671

Rubio R, Moraga E, and Borie F. 1990
Acid phosphatase activity and vesicular-arbuscular mycorrhizal infection associated with roots of 4 wheat cultivars
Journal of Plant Nutrition. **13**(5): 585–598

Sainz M J, Trasar M C, Arines, Gil-Stores F. 1987
Phosphatase activity in three acid soils as affected by VAM symbiosis and soil steam-sterilization
In *Proceedings of the Seventh North American Conference on Mycorrhiza*, 3–8 May 1987, Gainesville, Florida

Schubert A and Wiemken A. 1990
Dynamics of polyphosphate accumulation in extra- and intraradical hyphae of the VAM fungus *Glomus versiforme*
Innovation and Hierarchical Integration. Proceedings of the Eighth North American Conference on Mycorrhiza. 5–8 September 1990, Jackson, Wyoming, USA. p. 259

Sharma G D and Rao C S. 1987
Studies on CO_2 evolution and enzyme activities in root region of ectomycorrhizae of pine (*Pinus kesiya*).
Proceedings of the Seventh North American Conference on Mycorrhiza, 3–8 May 1987, Gainesville, Florida

Timonen S and Sen R. 1998
Heterogeneity of fungal and plant enzyme expression in intact Scots pine-*Suillus bovinus* and *Paxillus involutus* mycorrhizospheres developed in natural forest humus
New Phytologist **138**(2): 355–366

Tibbett M, Grantham K, Sanders F E, and Cairney J W G. 1998
Induction of cold active acid phosphomonoesterase activity at low temperature in psychrotrophic ectomycorrhizal *Hebeloma* spp.

Tillard P, Bousquet N, Mousain D, Martin F, Salsac L. 1990
Polyphosphatase activities in the soluble fraction of mycelil homogenates of *Pisolithus tinctorius*
Agriculture, Ecosystems and Environment, **28** (1–4): 525–528

Turnau K and Dexheimer J. 1995
Acid phosphatase activity in *Pisolithus arrhizus* mycelium treated with cadmium dust
Mycorrhiza **5**(3): 205–211

Research findings

Seasonal dynamics of vesicular arbuscular mycorrhiza and parasitic nematode *Helicotylenchus indicus* on phalsa

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Introduction

Phalsa (*Greia subinaequalis* D C) is a small, bushy, semi-arid plant grown throughout the country. The berry-like, globular, smooth, deep reddish brown (when ripe) summer fruit is a good source of phosphorus, iron, and vitamins A and C (Aykroyd

1963). It possesses high medicinal properties and exerts a cooling effect. In the Unani system of medicine, it has been recommended for the cure of fever, diarrhea, and heart ailments.

Phalsa is a drought tolerant plant, usually grown in sodic wastelands without fertilizer regimen. Adaptation to such hostile soils is due to mycorrhization as more than 80% of various plant species are reported to be mycorrhizal in nature (Gerdemann 1968). Phytoparasitic nematodes, similar to mycorrhiza, are of universal occurrence and inflict economic injuries to horticultural fruit plants, besides vegetables and other field crops (Sasser 1989). The damage caused by them is the function of their population density, which varies to a great extent with the seasons besides other factors. There is little information available on the population dynamics of VAM (vesicular arbuscular mycorrhiza) and parasitic nematodes on this crop with respect to seasons, and, therefore, monthly data was collected for mycorrhizal association, sporulation, and population density of the most predominant (80% frequency of occurrence) nematode, *Helicotylenchus indicus*.

Materials and methods

Site selection

Well-established (over 15 years old) phalsa orchards at the horticultural farms of the university were selected. The physico-chemical characteristics of the farm soil are as follows: silty clay loam, sticky and sodic in nature with pH (1:2)10.2, ECe (dS/m) 9.8, ESP 70, hydraulic conductivity (cm/hr) 0.04, gypsum requirement (me/100g soil) 8.2, organic carbon (%) 0.11, calcium carbonate (%) 4.5, and exchangeable cations (me/100g)-sodium 10.4, potassium 0.3, calcium 1.5 and magnesium 2.6 (Courtesy Department of Soil Science).

Sampling

Twenty-five soil and root samples from one hectare of phalsa fields were randomly collected in the last week of each month starting June 2003 through May 2004. These samples were processed in the laboratory as soon as possible or otherwise stored in a refrigerator at 10 °C.

Quantification of VAM colonization and sporulation

For spore density, soil samples were processed through the wet sieving and decanting techniques (Gerdemann and Nicolson 1963). For root colonization with VAM, thoroughly washed roots were stained in trypan blue after treating them in hot 10% potassium hydroxide aqueous solution (Phillips and Hayman 1970). The stained roots were cut into 1-cm segments, randomly picked up, and examined under stereomicroscope for mycorrhizal association. The root colonization was quantified following Nicolson's formula (1955):

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonized}}{\text{Total number of segments examined}} \times 100$$

Quantification of nematodes

Nematodes were extracted from thoroughly mixed 1-kg soil sample following Cobb's sieving and decanting, coupled with Baermann's funnel techniques. The measured volume of nematode suspension was taken into a counting dish and their number was counted under stereomicroscope.

Computation of data for seasonal effect

The effect of different seasons on VAM colonization, sporulation, and nematode population density was computed by calculating the average of concerned values of four months (June–September) for the rainy season, of five months (October–February) for the winter, and of three months (March–May) for the summer.

Identification of VAM fungi

The VAM species were identified on the basis of spore morphology following the manuals of Schenck and Perez (1987) and Mukerji (1996).

Results and discussions

It is evident from Table 1 that mycorrhizal colonization, sporulation, and *H. indicus* nematode population density increased from June through September 2003, decreased from October 2003 through January 2004, started to increase again from February 2004 through April 2004 (except nematode population which decreased), and finally decreased again during the month of May 2004. The highest amount of VAM colonization (63.2%) on phalsa root was observed during the month of April, sporulation (285.6 spores/100g soil) during September and *H. indicus* population density (335.3/100g soil) during August, whereas the lowest amount of colonization (12.5%), sporulation (21.5 spores/100g soil), and nematode population density (37.3/100g soil) was observed during the month of January 2004. It appears that seasons had a distinct impact on VAM and *H. indicus* nematode. Mycorrhizal colonization (50%), sporulation (201.1 spores/100g soil), and nematode density (244.3/100g soil) were the highest during the rainy season, followed with summer, and lowest (30%,102.1/100g soil, and 98.8/100g soil respectively) during the winter season (Figures 1–3).

Table1 Monthly changes in VAM colonization and sporulation and population density of *Helicotylenchus indicus* on phalsa

Month	*Root colonization* (%)	*Sporulation (spores/* 100 g soil)	*Nematode density* (No/100 g soil)
June	27.6 ± 5.3	69.0 ± 18.9	69.4 ± 20.8
July	52.0 ± 8.1	196.6 ± 43.9	303.6 ± 39.8
August	60.5 ± 8.1	253.2 ± 38.8	335.3 ± 60.2
September	59.8 ± 9.1	285.6 ± 44.6	268.8 ± 68.8
October	46.2 ± 7.9	251.2 ± 38.9	144.2 ± 47.9
November	35.4 ± 5.1	97.5 ± 12.8	125.6 ± 33.5
December	21.6 ± 8.0	53.9 ± 12.8	39.6 ± 10.7
January	12.5 ± 3.8	21.5 ± 6.2	37.3 ± 9.6
February	34.1 ± 8.6	86.6 ± 22.4	147.5 ± 30.4
March	53.4 ± 12.4	173.2 ± 39.0	285.7 ± 65.2
April	63.2 ± 7.9	206.6 ± 30.4	90.9 ± 21.8
May	22.0 ± 6.0	47.2 ± 11.1	64.4 ± 9.6

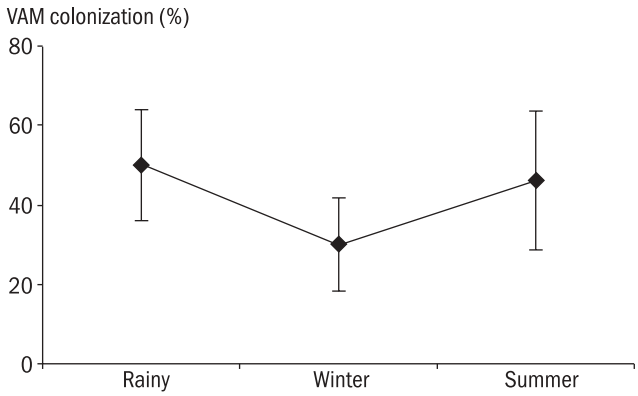

Figure 1 Effect of seasons on mycorrhizal colonization of phalsa roots

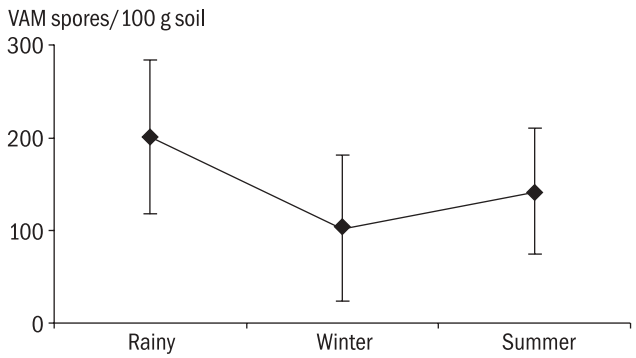

Figure 2 Effect of seasons on VAM sporulation

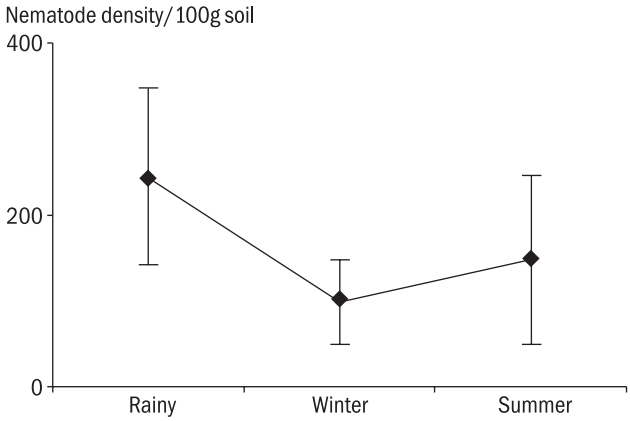

Figure 3 Effect of seasons on population density of *helicotylenchus indicus* on Phalsa

The highest amount of root colonization, sporulation, and *H. indicus* density during the rainy season could be attributed to the moderate climate, 90–95 relative humidity, and leaching of cations (sodium salts) (Mason, Musoko, Last 1992; Raghupathy and Mahadevan 1993) and also to better root growth, which provided ample entry points to VAM (Bhaskaran and Selvaraj 1997; Allen E B, Rincon, Allen M F, *et al.* 1998) and the feeding sites to the nematodes (Wallace 1973). With the onset of winter (October–February), phalsa enters in senescence stage due to the slowing down of metabolic activities, which adversely affect colonization and sporulation (Giovannetti 1985) and nematode activities (Norton 1978). With the onset of summer when senescence was broken down and plants rejuvenated due to the resumption of normal metabolism, new flushes came out and flowering set in. This coincided with qualitative and quantitative changes in root exudation, resulting in second peak population, though of a lower magnitude than was observed during the rainy season. Wallen (1980) and Gemma and Koske (1988) attributed this phenomenon to allocation of most of the photosynthate to roots and rhizome. VAM fungi appeared to be less diversified as only a few species such as *Glomus mosseae*, *G. fasciculatum*, *Gigaspora margarita*, *G. gigantea*, and *Acaulospora laevis* were encountered. Among these, *G. mosseae* was found to be the dominant species owing to its greater tolerance to soil alkalinity (Mosse 1973).

References

Allen E B, Rincon E, Allen M F, Perez-Jimenez A, Huante P. 1988
Distribution and seasonal dynamics of mycorrhiza in a tropical deciduous forest in Mexico
Biotropical **30**: 261–274

Aykroyd W R. 1963
The nutritive value of Indian foods and planning of satisfactory diets (6th ed.), ICMR, New Delhi, pp. 101–126

Bhaskaran C and Selvaraj T. 1997
Seasonal incidence and distribution of VA-mycorrhizal fungi in native saline soils
Journal of Environmental Biology **18**:209–212

Gemma J N and Koske R E. 1988
Seasonal variation in spore abundance and dormancy of Gigaspora gigt antea and in mycorrhizal inoculum potential of a dune soil
Mycologia **80**: 211–216

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

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

Giovannetti M. 1985
Seasonal variations of vesicular arbuscular mycorrhiza and endogonaceous spores in a maritime sand dune
Transactions of the British Mycological Society **84**: 679–684

Mason P A, Musoko M O, and Last F T. 1992
Short term changes in vesicular arbuscular mycorrhizal spore populations in Terminalia plantations in Cameroon. In: Mycorrhiza in Ecosystem (Read D J, Lewis D H, Hater H A, Alexander I J eds.), pp. 261–267, Cambridge University Press

Mosse B. 1973
Advances in the study of Vesicular-arbuscular mycorrhiza
Annual Review of Phytopathology **11**: 171–196

Mukerji K G. 1996
Taxonomy of endomycorrhizal fungi. In: Advances in Botany (Mukerji KG, Mathur B, Chamola B P, Chitralekha P eds), pp. 213–221 New Delhi: APH Corporation

Nicolson T H. 1955
The mycotrophic habit in grass
Nottingham, UK

Norton D C. 1978
Ecology of Plant-Parasitic Nematodes
New York: John Wiley & Sons

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

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

Sasser J N. 1989
Plant-parasitic nematodes: the former's hidden enemy
A Cooperative Publication of the Department of Plant Pathology and the Consortium for International Crop Protection, p. 115

Schenck N C and Perez Y. 1987
Manual for the identification of VA mycorrhizal fungi. P. 245, INVAM Florida, University of Gainesville, USA.

Wallace H R. 1973
Nematode Ecology and Plant Disease.
Edward Arnold.

Wallen B. 1980
Changes in structure and function of Ammophila during primary succession
Oikes **34**: 227–238.

Studies on arbuscular mycorrhizal fungi associated with *Jatropha curcas* L.

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While the diminishing of fossil fuel resources has become a major global concern, a well-known shrub, *Jatropha curcas* (Linn.), has the potential to become one of the world's key energy crops. It is commonly known as Ratanjot. Vegetable oil extracted from the seeds of *J. curcas* can be refined into bio-diesel for transport either in its pure form or as a blend with mineral diesel. In addition to this, its oil has various applications in the manufacturing of soaps, candles, lubricants, etc. Its

seed oil has medicinal values as well and is applied for rheumatism and skin disorders. *J. curcas* can also tolerate stress conditions because it requires minimum rainfall and can grow successfully on marginal, degraded, or even desert lands. This shrub species has therefore attracted the attention of farmers, politicians, industrialists, the government, and non-government organizations. Most of the organizations aim at multiplying this species and growing it in wastelands for want of its oilseeds. It has been well-established that colonization by AM (arbuscular mycorrhizal) fungi assures good survival and growth of plants on a variety of sites (Udaiyan, Sugavanam, and Manian *et al*. 1997; Kalawathi, Santhanakrishnan, Divya, *et al*. 2000; Vijay Kumar and Abraham 2001; Mamtha *et al*. 2003. AM fungi enhances the uptake of limited nutrients, hormone production, drought resistance, and suppression of root pathogens (Doss and Bagyaraj 2001).

In view of large scale afforestation of this species, there is need to develop quality seedlings for plantation and also to boost growth through the inoculation of AM fungi. In the present study, the status of AM fungi associated with *J. curcas* was investigated on two sites.

Material and methods

The existence of the AM–*Jatropha curcas* association was investigated in planted stands of *J. curcas* on the campus of the Tropical Forest Research Institute, Jabalpur, Madhya Pradesh. For this purpose, soil, along with fine feeder roots, was collected from the rhizosphere of the *J. curcas* plant, planted on two different soil types—black cotton soil (pH 7.5) and Red-murumy soil (pH 6.1). Isolation of AM spores was made by using the wet sieving and decanting techniques of Gerdemann and Nicolson (1963). Extraction of viable spores was worked out by the sucrose centrifugation technique of Daniel and Skipper (1982). Extracted spores were examined and their population was counted in nematode counting dishes under binocular research microscopes.

For the study of root colonization, fine feeder roots were collected and washed thoroughly in running tap water and 1 cm long segments were cut and stained in trypan blue following the method of Phillips and Hayman (1970). The root segments were observed under a compound microscope or hyphae. The percentage of root colonization was calculated by following the formula depicted below.

$$\text{Percentage of root colonization} = \frac{\text{Number of root segments with AM colonization}}{\text{Number of root segments observed}} \times 100$$

The identification of AM spores was done with the help of the manual prepared by Schenck and Perez (1987). The available nitrogen, phosphorus, and potassium were determined by following the methods of Jackson (1958). Isolation of *Azospirillum*, phosphate solubilizing bacteria, and fluorescent bacteria was made on specialized media.

Results and discussion

Observations regarding AM fungal spore population and the percentage of root colonization are presented in Table 1. Spore population and the percentage of occurrence of AM species in two different localities/soil types planted with *Jatropha curcas* varied significantly. Spore population in the rhizosphere of *Jatropha curcas* in black cotton soil was 328 spores per 100 grams of soil. However, red murumy soil showed less population with 207 spores per 100 grams of soil. Percentage of root colonization was also varied with 50% and 30% root colonization, respectively. Chandra and Jamaluddin (2004) found that the infection of VAM (vesicular arbuscular mycorrhiza) was enhanced by VAM inocula. As compared with other genera of AM fungi, genus glomus was the most dominant.

The available soil nutrients in black cotton soil were 147.4 kg/ha for nitrogen, 10.14 kg/ha for phosphorus, and 240 kg/ha for potassium. In red murumy soil it was 326 kg/ha for nitrogen, 22.15 kg/ha for phosphorus, and 216 kg/ha for potassium. The poor contents of phosphorus in black cotton soil enhanced the VAM spore population and its infection in roots.

Table 1 AM spores and its root colonization in *Jatropha curcas* in relation to nutrients level and soil reaction on two different sites

Soil type	*AM spore population* (per 100 g soil)	*Percentage of root colonization*	*Available N* (kg/ha)	*Available P* (kg/ha)	*Available K* (kg/ha)	*Soil reaction* (pH)
Black cotton	328	50	147.4	10.14	240.0	7.5
Red murumy	207	30	326.0	22.15	216.0	6.1

AM; g – gram; N – nitrogen; P – phosphorus; K – potassium; kg/ha – kilograms per hectare
– arbuscular mycorrhiza

Table 2 Occurrence of different AM Fungi in *Jatropha curcas* planted on two different sites

Name of AM species	*Percentage of occurrence*	
	Black cotton soil	*Red murumy soil*
Glomus aggregatum	33.3	39.0
Glomus macrocarpum	60.3	40.0
Glomus mosseae	80.0	56.8
Glomus fasciculatum	83.0	64.0
Glomus intraradices	64.5	35.0
Glomus etunicatum	28.6	40.0
Acaulospora scrobiculata	75.0	78.7
Acaulospora delicata	13.0	18.0
Gigaspora margarita	68.6	32.3
Scutellospora pellucida	33.5	28.5

In all ten species of AM fungi recorded in the soil of both sites under study (Table 2), the frequency of all the ten species varied considerably in both the localities. Other helper bacteria viz., *Azospirillum*, phosphate solubilizing bacteria, and fluorescent bacteria were also isolated from the rhizosphere of *J. curcas*.

AM fungi of genus *Glomus, Acaulospora, Gigaspora, and Scutellospora* were commonly found in both types of soil. *Glomus mosseae* was the dominant species to occur in the black cotton soil with 80% occurrence. The percentage of *Acaulospora delicata* was very poor in both the sites. In the case of red murumy soil, *Acaulospora scrobiculata* was found to be the dominant species with 78.7% occurrence. The pH value of black cotton soil was 7.5 and in case of red murumy soil it was 6.1. *J. curcas* is also stated to be significantly high mycorrhizal dependent (Anonymous 2005).

References

Anonymous. 2005
Mycorrizal technology: faster and environment - friendly method of growing *Jatropha curcas*, the bio-diesel plant.
Mycorrhiza News **17**(2): 15–16

Chandra K K and Jamaluddin. 2004
Effect of different mulches on effectiveness of VAM fungi and growth of *Albizia procera* in coal mine soil
Myforest **40**(3): 263–267

Daniel B A and Skipper H D.1982
Methods for the recovery and quantitative estimation of propagules from soil
In Schenck N C (ed.), Methods and principals of mycorrhizal research

Doss D D and Bagyaraj D J. 2001
Ecosystem dynamics of Mycorrhizae. Innovative approaches in microbiology
Maheshwari D K and Dubey R C (eds.) (2001) Dehradun: Bishen Singh Mahendra Pal Singh publishers, pp.115–129

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

Jackson M L.1958
Soil Chemical Analysis (Second ed)
New Delhi: Prentice Hall of India Pvt Ltd. pp. 498

Kalavathi B P, Santhanakrishnan K and Divya M P. 2000
Effect of VA-Mycorrhizal fungus and phosphorus solubilising bacterium in neem
Indian Forester **126**: 67–70

Mamtha G M S, Shivayogi D T, Bagyaraj D J, Suresh C K. 2003
Influence of the different VA isolates on growth and nutrition of sandalwood plants
My Forest **39**(2): 137–145

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

Schenck N C and Perez V. 1987
Manual for the identification of VA mycorrhizal fungi
Florida: Fifield Hall University Gainseville, pp. 241

Udaiyan K V, Sugavanam, and Manian S. 1997
Growth response of wattle (*Acacia mearnsii*) seedlings to phosphorus solubilization and inoculations with Glomus *deserticola* and Rhizobium sp. in non-sterile soil
J. Trop. Forest. sci., **10**: 212–224

Vijay Kumar K and Abraham T K. 2001
Mycorrhizal association of two endemic trees of western Ghats: *Gluta travancorica* and myristica malabarica
Indian Journal for Forestry **24**: 301–304

The growth and development of arbuscular mycorrhizal fungi and its effects on the growth of maize under different soil compositions

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Introduction

The importance of soil as a reservoir of plant nutrients responsible for primary productivity is well known; soil also provides the matrix for the biological processes involved in nutrient cycling. Among the biological processes involved in the rhizoplane, the unique role of symbiotic bacteria and the mycorrhizal fungi which ensure fixation and mobilization, and availability of nitrogen and phosphorus to plants have been well recognized (Sampathkumar and Ganeshkumar 2003). Mycorrhization is the characteristic feature of several agricultural crops (Muthukumar and Udaiyan 2002; Gupta and Baig 2001). The distribution of species of AM (arbuscular mycorrhiza) fungi varies with the climatic and edaphic environments, as well as with land use patterns (Singh 2000a; Hu Hung 1988). The effects of growing media, like soil, on the development of AM fungi and indirectly on the host plants are well documented (Davis, Young, and Linderman 1993; Singh 2000b). Amendments of soil with fungicides have also been reported to be inhibitory to the development of AM fungi (Robert, Peleger, Russell 1995; Anusuya and Dhaneshwari 1995). Similarly, the application of plant nutrient solution may also be helpful in the development of AM symbiosis (Schenck 1982). The present study is based on the effects of different soil compositions on AM fungi and maize hosts.

Materials and methods

Source of AM spores

The AM spores belonging to *Glomus* sp. were isolated from the rhizosphere soil of tea (*Camelia sinensis* L.) plantations of the Bhuyanpirh tea estate of Orissa Tea Plantations Ltd situated in Tarmakanta, about 48 km away from the city of Keonjhar, Orissa. The plantation was placed in an area that was once covered by dry and mixed deciduous sal forests at an elevation of more than 600 m. The soil was red clay-loam and poor in nutrient content.

Isolation of Glomus *sp.*

The isolation of AM spores was carried out following the method of Gerdemann and Nicolson (1963). The representative soil samples (100 g) were suspended in sufficient quantities of water and stirred thoroughly. The resulting soil suspension was sieved through mesh sizes 400, 300, 200, and 100 µm, and placed one below the other in the same order. The residues left after sieving were filtered through whatman paper (No. 1) and observed under a stereo zoom microscope (Meiji, Japan) for spore count. The soil pH was measured by a Mettler Toledo (MA 235 pH/ion r) pH meter.

Identification of AM fungal spores

The isolated spores were given a thorough microscopic examination to record their morpho-taxonomic features. Preliminary observations helped to segregate them differently on gross morphology. Later, diagnostic slides were prepared according to the INVAM guidelines (Schenck and Perez 1987) and tentative identification was made as per the manual of AM fungi.

Pure culture preparation of AM fungi

AM spores obtained from the tea plantation rhizosphere soils were purified following the funnel technique (Menge and Timmer 1982) for which single spore isolation was done by picking AM spores from the spore mass left on the sieves. A device was made by keeping a funnel on the bottle (filled with nutrient solution). This funnel was first filled with small amount of sterilized sand and soil mix (1:1) up to the neck portion. At the neck portion of funnel, individual spores of AM fungi were kept and filled with the sand-soil mix. Maize seeds were sown under controlled conditions in a glasshouse and their roots were allowed to come into contact with spores through neck portion. During the growing one-month period, daily watering was done and weekly nutrient solution (1/4 strength Hoagland) was added. Afterwards, seedling roots were analysed for colonization of AM fungi. The complete system, including soil and seedlings, were transferred into the bigger earthen pots containing sterilized sand soil and soilrite mix for the multiplication of individual spores.

Multiplication and maintenance of AM fungi

The pure culture of isolated AM fungi was used for pot culture inoculations of rice, onion, and maize

as the hosts for their multiplication. During multiplication, host plants were nourished by Hoagland nutrient solution quarterly and daily watering was done up to three months.

Experimental set up

An experiment was carried out to determine the multiplication status of AM spores in host plants. The experiment was set with sterilized soil in specific composition under glasshouse conditions. Seedlings were raised in the earthen pots with 5-kg capacities. The entire experimental set was divided into following groups where 15-day old maize seedlings were selected as host plants.

The soil inoculum containing 250 spores/100g soil was used in this study. Fungicides – that is, bavistin and indofil – were added to the soil at the rate of 0.01%/25 ml/pot/fortnight. Nutrient solution was added in ¼ strength, 100 ml/pot/ week. Plants with all the treatments were grown in pots containing 5 kg of the abovementioned composition and were watered daily. The maize plants were uprooted and measured for growth parameters at an interval of 30 days up to 75 days of growth period. Final observations were taken on wet and dry biomass, plant height (shoot and root length), leaf number, and mycorrhization in terms of percentage of colonization, spore count, and vesicle number in the plants of all soil compositions. Data obtained in triplicate were subjected to the statistical analysis (Sokal and Rohlf 1973).

Microscopic examination of maize roots

Plants (150 day old) were harvested and roots were treated for clearing and staining following the method of Phillips and Hayman (1970) to determine the AM colonization in the root systems of individual plants. Five plants were studied under each treatment. Root samples were cleaned by 10% potassium hydroxide and autoclaved for 15–20 minutes at 15 lb/inch pressure; the autoclaved root samples were treated with 6 N hydrochloric acid for 5 minutes. The cleared roots were then stained with 0.05% cotton blue. The percentage infection was calculated by the function of number of root bits infected to the number of root bits observed. The analysis of percentage of colonization in the roots of different plants was done according to slide method (Kormaink and McGraw 1982). Besides percentage colonization, roots were observed to record the number of vesicles and their shape. Microphotography was done for the documentation of colonization patterns with the help of a Stereozoom microscope (Nikon Optiphot).

Determination of growth parameters

Simple biological norms were taken into consideration for the determination of growth parameters including shoot height, root length, leaf number, leaf area, wet and dry biomass of shoot, root, and leaf, shoot and root ratio.

Determination of AM spore-count

The rhizosphere soil of maize grown under different treatments was treated for the AM spore isolation and the total number of spores present in 100 g of soil was counted simply with the help of stereo zoom microscope.

Results

(i) Pattern of mycorrhization in maize

Maize plants grown under different soil compositions inoculated with AM fungi showed mycorrhization in their roots, where as uninoculated plants had no mycorrhization (Figure 1). Plants of black soil, sand, and compost (T2) (2:1:1) showed 84% colonization followed by T3 (42%), T7 (39.97%) and T6 (37.33%). The other two soil compositions had poor AM colonization—that is, T4 (14.33%) and T5 (5.67%). Analysis of

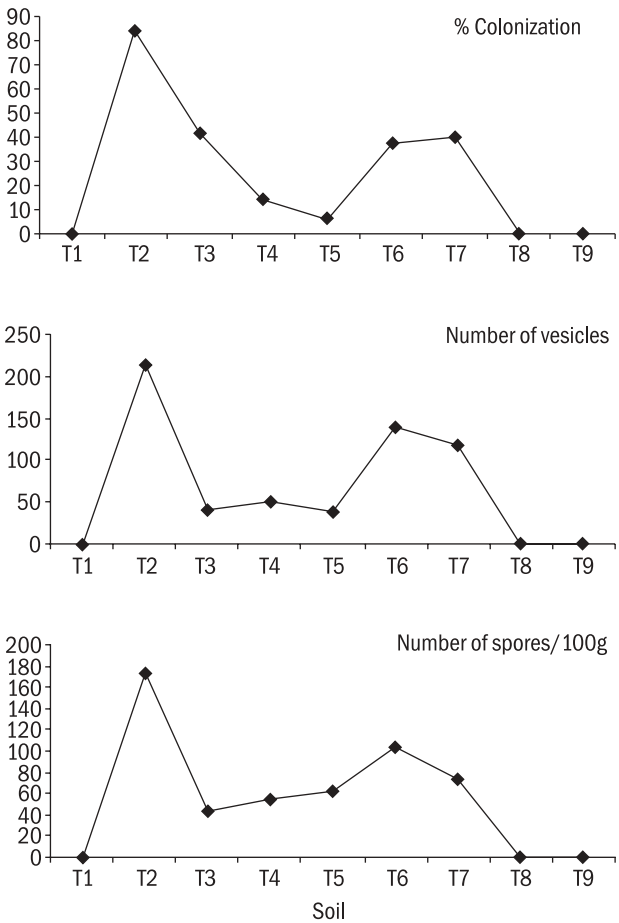

Figure 1 Status of mycorrhization in maize roots inoculated under different soil compositions

mycorrhization in fungicide treated soil and plants did not show any colonization in their roots.

The total number of vesicles in the infected roots was also calculated and it was higher in T2 (214.67) followed by T6 (138.33) and T7 (117). Data obtained for the total spore count present in the rhizosphere showed the highest value in T2 (173.33/100 g soil) followed by T6 (103.33) and T7 (73.33). Analysis of spore counts in the soil T8 and T9 added with fungicide did not show any spores in the rhizosphere soil.

(ii) Growth performance of maize under mycorrhization

An experiment was carried out to determine the efficiency of AM fungi under different soil compositions. The response of host plants towards mycorrhization by the indigenous fungi was evaluated by measuring the growth parameter such as fresh and dry biomass, plant height, leaf number, and mycorrhization in terms of percentage of colonization, spore count, and vesicle number.

The data obtained at an interval of 15 days and at the final stage of 75 days of growth are presented in Tables 1 and 2 and Figures 2, 3, and 4. Periodical analysis of plant growth showed a gradual increment in plant height and leaf number. AM inoculated plants induced better growth in maize plants as compared to the uninoculated ones. T2 type soil (that is, black cotton soil + sand + compost [2:1:1]) and T7 (that is, black cotton soil + sand + compost [2:1:1]) added with Hoagland nutrient solution showed higher plant height (78.87 and 77.33 cm, respectively) under

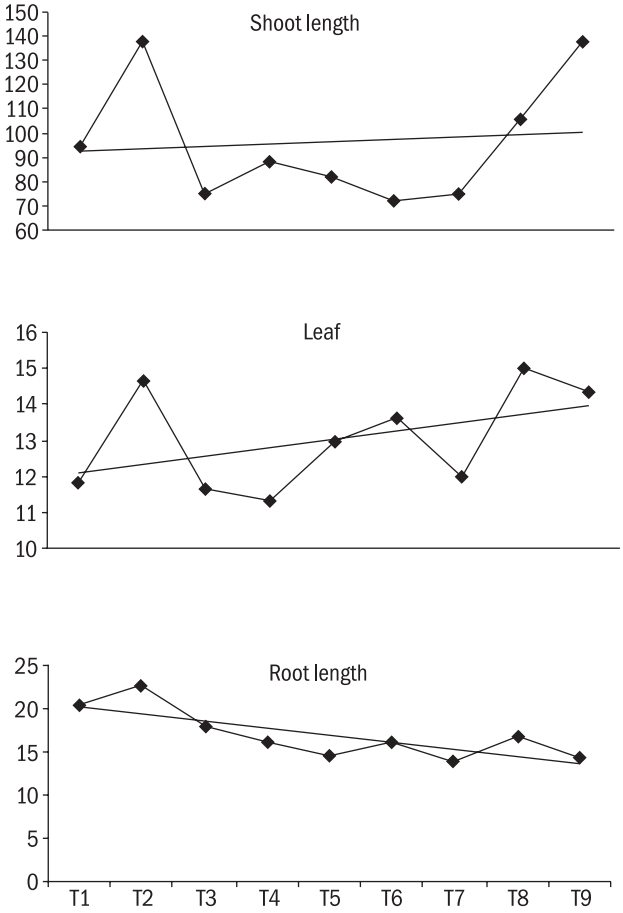

Figure 2 Effect of AM fungi on growth of maize grown under different soil compositions (measured after 75 days of experiment)

Table 1 Effect of AM fungi on growth of maize inoculated in different soil compositions (periodical analysis)

	Shoot length			*Number of leaves*			*Leaf area cm² (average)*		
Treatments	*30*	*45*	*60*	*30*	*45*	*60*	*30*	*45*	*60*
T1	20.60 ± 0.37	40.97 ± 1.43	55.35 ± 2.78	6.70 ± 0.19	9.50 ± 0.29	11.00 ± 0.0	71.89	170.8	171.09
T2	21.11 ± 0.65	37.36 ± 1.1	78.87 ± 3.2	6.48 ± 0.15	9.25 ± 0.48	10.00 ± 0.0	41.93	314	274.73
T3	22.95 ± 0.85	42.20 ± 2.15	60.53 ± 4.67	7.25 ± 0.25	9.50 ± 0.29	11.25 ± 1.18	42.1	103.3	208.36
T4	26.94 ± 1.06	45.70 ± 4.27	68.78 ± 5.28	7.25 ± 0.25	8.40 ± 0.68	10.40 ± 0.24	33.53	190.35	279.11
T5	24.48 ± 0.74	44.33 ± 3.38	71.73 ± 4.45	7.25 ± 0.25	9.50 ± 0.5	12.00 ± 0.71	55.74	205.27	223.33
T6	23.08 ± 1.44	42.06 ± 4.06	61.42 ± 6.89	7.20 ± 0.37	10.00 ± 0.0	11.00 ± 0.0	38.43	184.75	165.24
T7	23.22 ± 1.28	42.63 ± 3.63	65.18 ± 5.8	7.17 ± 0.17	9.83 ± 0.31	11.83 ± 0.4	64.23	156.81	256.58
T8	24.01 ± 1.17	47.30 ± 3.12	75.01 ± 5.22	7.25 ± 0.37	9.33 ± 0.33	11.00 ± 0.44	30.9	101.77	194.67
T9	24.41 ± 0.49	44.83 ± 2.66	77.33 ± 2.32	7.25 ± 0.25	9.40 ± 0.4	13.00 ± 0	88.58	151.81	224.33

T1 Black cotton soil + sand + compost (2:1:1)
T2 Black cotton soil + sand + compost (2:1:1) + AM fungi
T3 Black cotton soil + AM fungi
T4 Black cotton soil + sand (1:1) + AM fungi
T5 Black cotton soil + soilrite (1:1) + AM fungi
T6 Black cotton soil + sand + soilrite (1:1:1). + AM fungi
T7 Black cotton soil + sand + compost (2:1:1)+ hogland nutrient+AM fungi
T8 Black cotton soil + sand + compost (2:1:1) + Bavistin + AM fungi
T9 Black cotton soil + sand + compost (2:1:1) + indofil + AM fungi

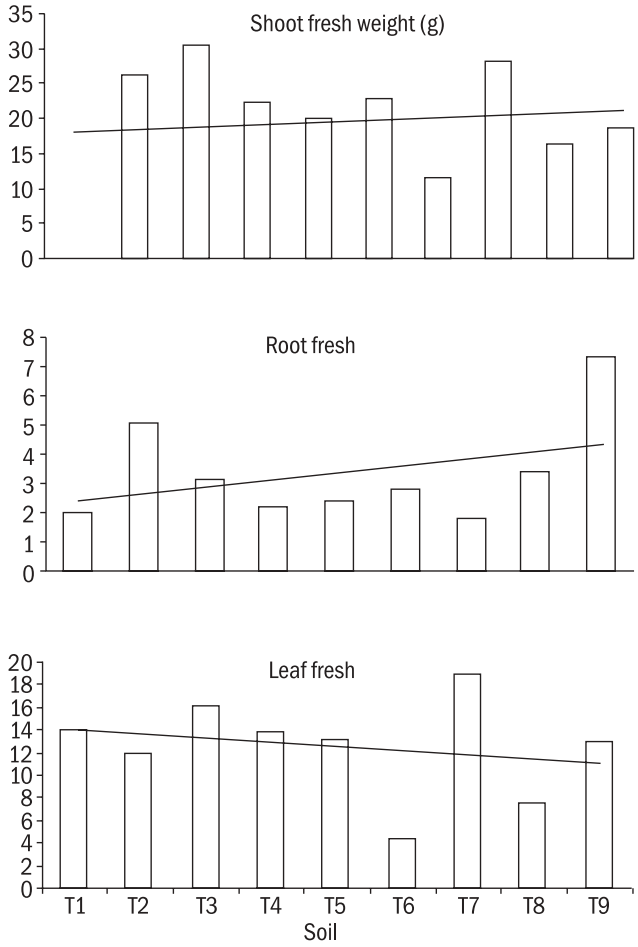

Figure 3 Effect of AM fungi on growth of maize grown under different soil compositions (measured after 75 days of experiment)

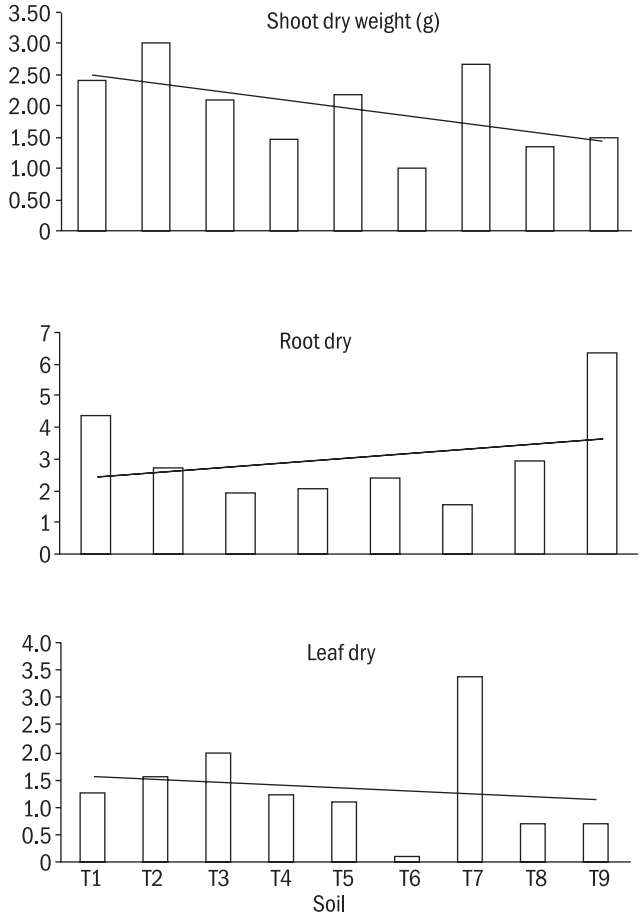

Figure 4 Effect of AM fungi on growth of maize grown under different soil compositions (measured after 75 days of experiment)

mycorrhizal inoculation, followed by T5 (black cotton soil + soilrite [1:1], 71.73 cm). The total number of leaves was recorded to be higher in plants grown in treatment T9 (13) and T5 (12). Plants under other treatments had 10–11 leaves at 60 days. Average leaf area and total leaf area were also calculated during this experiment. The value was found to be higher in T4 (279.11 cm^2 per leaf) followed by T8 (256.58 cm^2 per leaf).

At the final stage (75 days), mycorrhizal treatment proved to enhance the growth of maize plants as compared to the uninoculated control (Table 2). Data recorded for plant height – that is, shoot length – showed the best performance of plants grown under T2 (137.78 cm) AM fungi followed by T7 (138.13 cm) (Figure 2). Plants of all mycorrhizal treatments of all soil types showed 13–15 leaf numbers except T1, T3, and T4 where only 11 leaves were observed (Figure 2). The maximum root length was observed in T2 type soil followed by T1. Plants of other soil types did not show any significant variation among them except T8 soil type. The highest fresh and dry shoot weight was obtained in T2 followed T7 for root biomass and T8 for leafy biomass (Figures 3 and 4). However, a varied performance of maize was observed in different soil compositions as far as the root and leaf dry biomass was concerned.

(iii) Shoot–root ratio

Shoot–root ratio with respect to length was higher in T9 (6.09) followed by T8 and T2 (Figure 5). Biomass with regard to the yield, shoot–root ratio was found to be higher in T7.

(iv) Proportional distribution of biomass

Proportional distribution of biomass covered by different parts of maize plants grown under uninoculated and inoculated conditions and different soil compositions were analysed (Figure 6). The maximum biomass was covered by root of inoculated plants except the plants grown under T2 and T5, where 31% and 39% biomass was assigned to the shoots, respectively. Similarly, uninoculated control plants also showed root biomass proportionally higher than shoot and leaf.

Table 2 Effect of AM fungi (isolated from tea plantation) on growth (measured after 75 days of experiment) of maize (host plant) grown under different treatments (shoot length, root length, leaf number)

code	*SL*	*LN*	*RL*	*SFW*	*RFW*	*LFW*	*SDW*	*RDW*	*LDW*
T1	95.07 ± 7.27	11.83 ± 0.65	20.41 ± 0.95	26.25 ± 1.49	2.03 ± 0.28	11.94 ± 0.84	2.42 ± 0.21	1.76 ± 0.28	1.55 ± 0.20
T2	137.78 ± 7.80	14.66 ± 0.33	22.62 ± 0.86	30.35 ± 1.38	5.05 ± 0.69	14.1 ± 0.79	3.01 ± 0.22	4.38 ± 0.69	1.27 ± 0.16
T3	74.89 ± 12.70	11.66 ± 1.67	17.90 ± 0.95	22.35 ± 1.78	3.16 ± 0.20	16.17 ±0.53	2.09 ± 0.18	2.74 ± 0.20	1.98 ± 0.03
T4	88.32 ± 6.90	11.33 ± 0.67	16.16 ± 0.54	20.07 ± 1.47	2.21 ± 0.13	13.91 ±0.43	1.46 ± 0.33	1.92 ± 0.13	1.22 ± 0.10
T5	81.90 ± 9.28	13.00 ± 1.00	14.50 ± 0.64	22.73 ± 2.34	2.41 ± 0.29	13.15 ±0.54	2.18 ± 0.21	2.09 ± 0.29	1.08 ± 0.05
T6	72.07 ± 755	13.66 ± 0.67	16.13 ± 0.67	11.48 ± 1.04	2.78 ± 0.17	4.33 ± 0.55	1.01 ± 0.23	2.41 ± 0.17	0.10 ± 0.02
T7	74.70 ± 6.74	12.00 ± 0.00	13.86 ± 0.54	28.35 ± 0.92	1.82 ± 0.18	18.93 ±1.47	2.66 ± 0.31	1.58 ± 0.18	3..37 ± 0.28
T8	106.33 ± 11.93	15.00 ± 0.00	16.83 ± 0.80	16.40 ± 0.93	3.4 0 ± 0.52	7.55 ±0.79	1.34 ± 0.17	2.95 ± 0.52	0.69 ± 0.16
T9	138.13 ± 13.43	14.33 ± 0.67	18.56 ± 1.88	18.57 ± 1.83	7.34 ± 1.17	13.04 ±4.92	1.49 ± 0.26	6.37 ± 1.17	0.70 ± 0.21

SL – shoot length; LN – leaf number; RL – root length; SFW – shoot fresh wt.; RFW – root fresh wt.; LFW – leaf fresh wt.; SDW – shoot dry wt.; RDW – root dry wt.; LDW – leaf dry wt.; ± – standard error of mean; n – 3

T1 Black cotton soil + sand + compost (2:1:1)
T2 Black cotton soil + sand + compost (2:1:1) + AM fungi
T3 Black cotton soil + AM fungi
T4 Black cotton soil + sand (1:1) + AM fungi
T5 Black cotton soil + soilrite (1:1) + AM fungi
T6 Black cotton soil + sand + soilrite (1:1:1). + AM fungi
T7 Black cotton soil + sand + compost (2:1:1)+ hogland nutrient + AM fungi
T8 Black cotton soil + sand + compost (2:1:1) + Bavistin + AM fungi
T9 Black cotton soil + sand + compost (2:1:1) + indofil + AM fungi

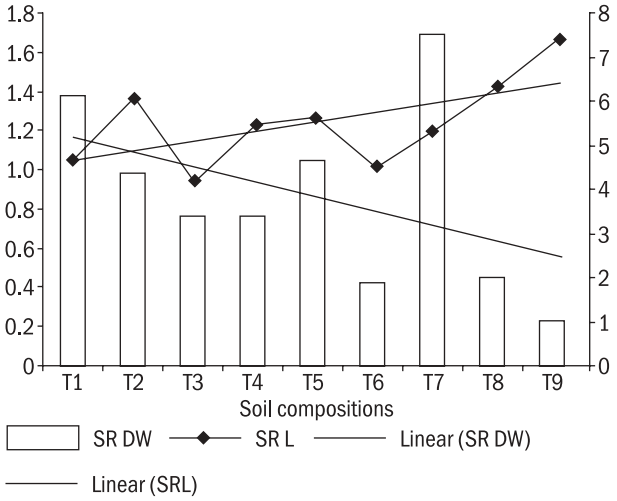

Figure 5 Shoot / Root ratio in maize grown under mycorrhization and different soil compositions

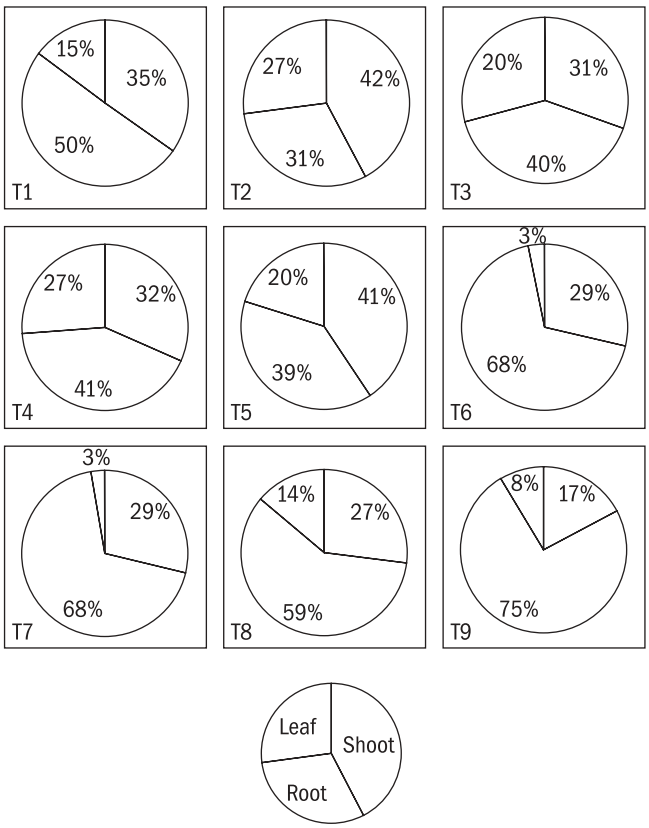

Figure 6 Proportional distribution of dry biomass in maize grown with AM fungi in different soil compositions

(v) Statistical analysis

Among the treatments experimented, different soil compositions, and soil added with nutrient solution and fungicides application was found significantly variable at 1% level and 0.1% level for the shoot length (Table 3).

Analysis of variance for leaf number of different stages of maize growth under different treatments (Table 4) revealed that the variation in the periodical effect of soil composition was significant at 5% level only. On the other hand, the rest of the treatments varied significantly (0.1% level) with respect to maize growth development and growth under different treatment studied.

A wide level of variation was found through the analysis of variance for leaf area at different stages of maize growth under different treatments, which is shown in Table 5. Among the treatments, all except nutrient application and fungicide application showed significant variations at 0.1% level.

Data recorded for root length and root dry biomass and percentage of colonization was also subjected to statistical analysis (Table 6). It was observed that root length and root dry biomass were positively correlated with percentage of colonization in maize plants of different soil compositions. However, data analysed for correlation between colonization and number of spores and/or vesicles seemed to be not correlated. Similarly, vesicle number and spores were found to be significantly correlated.

Discussion

In the present study, mycorrhization in maize plants grown in different types of media exhibited the adaptability of indigenous mycorrhizal fungi and host acceptability for multiplication and development. This corroborated the findings of Gardezi, Jean, Plazola, *et al.* (1990) who reported the comparative effectiveness of *Glomus* in bromide soil compared to clay loam soil. Present data also exhibited the varied percentage of colonization of infected roots grown in different soil types. The existence of a varied range of colonization might be attributed to the soil factor which affected the number of vesicles/root and spores in the rhizosphere soil. Although mycorrhization occurred in the inoculated plants, no correlation could be established between the spore number and vesicle number. However, number of vesicles were found to be significantly correlated with spore number/ root. Soil texture may affect plant growth besides mycorrhizal efficiency (Tacon, Kabre, Garbaya *et al.* 1979). The investigation revealed that red soil mixed with sand and compost was the best in enhancing plant growth. The increases in plant biomass and height due to mycorrhizal fungi were also reported widely (Ikram, Ghani, Ibrahaim, *et al.* 1992). Black cotton soil alone did not affect the mycorrhizal efficiency in improving plant growth; a better performance was achieved when mixed with sand, soilrite, and red soil composition. This result was supported by the views of Nence (1987) who stated the superiority of perlite sand mix over only sand. However, the addition of sand was found to be advantageous as the greatest dry weight was

Table 3 ANOVA for shoot length at different stages of growth of maize under different treatments

Treatments	*Source*	*SS*	*DF*	*MS*	*F*
Soil compostion	Treatment (between time)	15730	3	5242	32.66 ***
	Residual (within treatment)	3211	20	160.5	
	Total	18940	23		
Soil compostion with nutrient	Treatment (between time)	10950	3	3651	13.1**
	Residual (within treatment)	2229	8	278.7	
	Total	13180	11		
Soil compostion with fungicides	Treatment (between time)	21090	3	7029	46.43***
	Residual (within treatment)	1817	12	151.4	
	Total	22910	15		

SS – sum of squares; DF – degrees of freedom; MS – mean square

Table 4 ANOVA for leaf number at different stages of growth of maize under different treatments

Treatments	*Source*	*SS*	*DF*	*MS*	*F*
Soil compostion	Treatment (between time)	104.6	3	34.86	53.65*
	Residual (within treatment)	13	20	0.6498	
	Total	117.6	23		
Soil compostion with nutrient	Treatment (between time)	58.46	3	19.49	21.74***
	Residual (within treatment)	7.171	8	0.8964	
	Total	65.63	11		
Soil compostion with fungicides	Treatment (between time)	106.2	3	35.4	36.93***
	Residual (within treatment)	11.5	12	0.9586	
	Total	117.7	15		

SS – sum of squares; DF – degrees of freedom; MS – mean square

Table 5 ANOVA for average leaf area at different stages of growth of maize under different treatments

Treatments	*Source*	*SS*	*DF*	*MS*	*F*
soil compostion	Treatment (between time)	104700	2	52350	21.57***
	Residual (within treatment)	36410	15	2427	
	Total	141100	17		
soil compostion with nutrient	Treatment (between time)	54940	2	27470	7.614*
	Residual (within treatment)	21650	6	3608	
	Total	76590	8		
soil compostion with fungicides	Treatment (between time)	55830	2	27910	7.617*
	Residual (within treatment)	32980	9	3665	
	Total	88810	11		

SS – sum of squares; DF – degrees of freedom; MS – mean square

found due to this treatment (Menge, Labanauskas, Johanson, *et al.* 1979).

The addition of compost was reported for the enhancement of spore population (Muthukumar and Udaiyan 2002), which was evident by the performance of AM fungi in T2 soil. The pH of the soil was acidic. The incidence and development of fungi in the acidic soil exhibited their ability to tolerate such adverse condition. However, several other reports were available on the occurrence of AM

Table 6 Correlation coefficient of % colonization and vesicles (no. of maize grown under different soil compositions)

I variable	*II variable*	*R squared*	*P value*
%colonization	root length	0.9221	**
%colonization	root dry biomass	0.926	**
%colonization	spores	0.6828	ns
%colonization	vesicles	0.7364	ns
vesicles	spores	0.9633	**

** – significant at 1% level; ns – non significant

at low pH (Gupta and Krishnamurthy 1996). Davis, Young, Lindermann, *et al.* (1993) reported enhancement in the growth of host plants with different degrees depending upon the apparent pH tolerance of AM fungi. It is important to note that root dry biomass and root length was positively correlated with the percentage of colonization of the roots. It was reported that AM inoculation resulted in the increase of root length and hence root dry weight (Tholkappian, Sathiyavathy, Sundram 2000).

In the present study on the inoculation of AM fungi on maize, grown with different soil compositions, variations in the degree of symbiosis and the consequent benefit were clearly evident. Soil texture might affect plant growth as well as mycorrhizal efficiency in various ways including drainage, limiting nutrient availability and aeration, etc. Addition of sand and soilrite enhanced the porosity of soil and compost provided the required nutrients. In such conditions, the inoculation of AM fungi helped in the enhancement of plant productivity.

Poor plant growth as well as AM colonization in the present study was contrary to the findings of Shenck (1982) who reported that a modified Hoagland solution devoid of phosphorus could provide a planting medium conducive to the development of mycorrhizal symbiosis. Since a low supplementary dose of specific elements might be needed for plants with special nutritional needs, a standardization of the specific nutrient requirement of maize and AM association might be a prerequisite to reach any conclusion.

In the present study, the complete inhibition of AM colonization in the roots of maize treated with fungicide was observed and the results confirmed that fungicides inhibited hyphal development (Graham, Timmer, Fradelmann, *et al.* 1986). Sukarno *et al.* (1996) reported the effect of the fungicides on mycorrhizal symbiosis and correlated with phosphorus uptake. Similar effects were reported by Anusuya and Dhaneswari (1995) who stated that the AM infection was inhibited by the application of different types of fungicides. This was also corroborated by Robert, Pelger, Russelle, *et al.* (1995) who envisaged the merit of fungicide application in stimulating the colonization and spore production by AM fungi. This was consistent with the report of Anusuya (1995) who reported an increase in fungal infective propagules with fungicide application. It was also reported that at low concentrations, fungicide had no adverse effects (Vyas, Vyas, Mahajan, *et al.* 1990). The complete suppression of AM infection and colonization in the present study might be due to the high concentration and type of fungicides used. The enhanced growth of maize due to the application of fungicide might be because of the elimination of harmful fungi from the rhizosphere. However, assessment of the effects of fungicides on the responses of mycorrhiza might be useful to develop a package of practices for agricultural and horticultural crops.

Acknowledgements

The authors are thankful to the Department of Forest and Environment, Government of Orissa for their help.

References

Anusuya D. 1995
Interactions of carbendazim and vesicular arbuscular mycorrhizal fungi on growth and yield of chilli (*Capsicum annum* L.)
In: Mycorrhiza biofertiliser for the future Proceedings of the third national conference on mycorrhiza. Eds. Adholeya A and Singh S, pp.144–146

Anusuya D and Dhaneswari D. 1995
Effects of fungicides on nodulation and vesicular arbuscular mycorrhizal development in groundnut
In: Mycorrhiza biofertiliser for the future Proceedings of the third national conference on mycorrhiza. Eds. Adholeya A and Singh S, pp. 141–143

Davis E A, Young J L, and Lindermann R G. 1993
Soil lime level pH and vesicular arbuscular mycorrhizal effects on growth responses of sweet gum, liquidamber styraciflua seedlings
Soil Science Society of American Journal **47**(2): 251–256

Gardezi A K, Jaen C D, Plazola G, Ferrera R A, Cerrto R. 1990
Growth of Acacia farnesiana associated with mycorrhizal fungi in three types of Mexican soils
Nitrogen Fixing Tree Research Reports **8**: 99–102

Gerdemann J W and Nicolson T H. 1963
Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting
Transaction of British Mycological Society **46**(2): 235–244

Graham J H, Timmer L W, and Fradelmann D. 1986
Toxicity of fungicidal copper in soil to citrus seedlings and vesicular arbuscular mycorrhizal fungi
Phytopathology **76**: 66–70

Gupta N and Baig S. 2001
Evaluation of synergistic effect of phosphate solubilising Penicillium spp., AM fungi and rock phosphate on growth and yield of wheat *Philippines Journal of Science* **130**(2): 131–135

Gupta R and Krishnamurthy K V. 1996
Response of mycorrhizal and non mycorrhizal Arachis hypogeae to NaCl and acid stress
Mycorrhiza **6**: 145–149

Hu Hung. 1988
Studies on the symbiotic relationship between truffles and trees (III). Effect of soil characteristics on the mycorrhiza formation.
Quarterly *Journal of Chinese Forestry* **21**(4): 111–118

Ikram Mahmud A W, Ghani M N, Ibrahim M T and Zainal A B. 1992.
Field nursery inoculation of Heavea brasiliensis Muell. Arg. seedling root stock with vesicular-arbuscular mycorrhizal fungi
Plant and Soil **145**(2): 231–236

Kormanic P P and Mc Graw A C. 1982
Methods and principles of mycorrhizal Research.
Ed. N C Schenck
American Phytopathological Society, pp. 34–45

Menge J A, Labanauskas C K, Johanson E L V, Sibert D. 1979
Problems with the utilization of mycorrhiza fungi in the production of containerized citrus in the nursery or green house. In: Proceedings of the fourth North American conference on mycorrhiza, Colorado, USA

Menge J A and Timmer L M. 1982
Procedure for inoculation of plants with vesicular arbuscular mycorrhiza in the laboratory, green house and field
Methods and principle of mycorrrhizal research, ed. N C Schenck. American Phytopathological Society, pp. 59–68

Muthukumar T and Udaiyan K. 2002
Growth and yield of cowpea as influenced by changes in arbuscular mycorrhiza in response to organic manuring
Journal of Agronomy and Crop Science **188**(2): 123–132

Nence S. 1987
Citrus seedlings growth and Glomus intraradices infection as affected by various soil-free potting media. In: Mycorrhiza in the next decade: practical applications and research priorities, edited by D M Slyvia, L L Hung, and J H Graham. Proceedings of the seventh North American conference on Mycorrhiza, 3–8 May 1987, Gainesville, Florida. pp.364.

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

Robert N, Peleger F L, and Russelle. 1995
Interactions between native prairie grasses and indigenous arbuscular mycorrhizal fungi implications for reclamation of taconite iron ore tailing
New Phytologist **129**: 651–660

Sampathkumar G and Ganeshkumar A. 2003
Effect of AM fungi and Rhizobium on growth and nutrition of *Vigna mungo* L., and *Vigna unguiculata* L.
Mycorrhiza News **14**(4): 15–16

Schenck N C. 1982
Methods and principles of mycorrhizal research.
American Phytopathological Society, pp. 37–45

Schenck N C and Perez Y. 1987
Manual for the identification of VA mycorrhizal fungi, INVAM, Florida, 245.

Singh S. 2000a
Mycorrhiza and soil organic amendments
Mycorrhiza News **12**(2): 2–12

Singh S. 2000b
Effect of edaphic and climatic factors on the development of mycorrhiza in tree nurseries (part II). Effect of soil pH, light and carbon dioxide
Mycorrhiza News **11**(4): 3–13

Sokal R R and Rohlf J F. 1973
Introduction to biostatistics
San Francisco: Freeman

Sukarno N, Smith F A, Smith S E, Scott E S. 1996
The effect of fungicides on vesicular arbuscular mycorrhizal symbiosis II. The effect on area of interface and efficiency of P uptake and transfer to plant
New Phytologist **132**: 583–592

Tacon F L E, Kabre A, Garbaya J. 1979
Influence of endomycorrhizae on mineral nutrition of Acer Pseudoplatanus. In: proceedings of Fourth North American Conference on mycorrhiza, Colorado, USA.

Tholkappian P, Sathiyavathy R J, Sundram M D. 2000
Mycotrophy of sweet potato (*Ipomoea batata*) in coastal soils of Tamil Nadu
Mycorrhiza News **12**(2): 13–14

Vyas S C, Vyas A, Mahajan K C, and Shroff V N. 1990
Effect of fungicides on mycorrhizal and Rhizobium development in soybean. In: Current trends in mycorrhizal research, eds. B L Jalali and H Chand. Proceedings of the National Conference on Mycorrhiza, Hisar, pp 14–16

New approaches

Characterization of AM fungi on roots from the field

Identification of AMF (arbuscular mycorrhizal fungi) on roots is almost impossible with morphological methods and, due to the presence of contaminating fungi, it is also difficult with molecular biological techniques. To allow broad investigation of the population structure of AMF in the field, Rankar C, Heinrichs J, Kaldorf M, and Buscot F (2003) established a new method to selectively amplify the ITS (internal transcribed spacer) region of most AMF with a unique primer set (Mycorrhiza 13(4): 191–198, 2003). Based on the available sequences of the rDNA, one primer pair specific for the AMF and a few other fungal groups was designed and combined in a nested PCR with the already established primer pair ITS 5/ITS 4. Amplification from the contaminating organisms was reduced by an AluI restriction after the first reaction of the nested PCR. The method was assessed at five different field sites representing different types of habitats. Members of all major groups within the Glomeromycota (except Archaeosporaceae) were detected at the different sites. Gigasporaceae also proved detectable with this method based on cultivated strains.

Centre for Mycorrhizal Culture Collection

Screening of *Jatropha curcas* germplasm from different provenances for cultivation in fly ash overburdens using arbuscular mycorrhiza fungi

Reena Singh, Mahaveer P Sharma, and Alok Adholeya

The safe disposal and effective and economic utilization of fly ash voluminously generating from various coal-based thermal power stations and strict compliance of the environmental regulation are of immense concern. Mycorrhizal fungi, through their mycelial network, accumulate heavy metals from fly ash and retain them within their cells or carry them on their body surface when they form association with the plants. These mycelial threads, along with dense root biomass, assist in binding ash particles. *Jatropha curcas* is itself a very hardy plant and with its known naturally existing association with mycorrhizal fungi, the mycorrhized jatropha plantations will not only produce higher yields with respect to seed production, but will also give a higher vegetative biomass.

The present study was thus undertaken with the objective of establishing and screening various genotypes of *Jatropha curcas*, preinoculated with selected AMF (arbuscular mycorrhizal fungi) using fly ash as the medium in a nursery.

Methodology

Collection of germplasm and multiplication
Jatropha curcas was collected from the following places.

- Sehore, Madhya Pradesh
- Beed/Kollar, Khandwa, Madhya Pradesh
- Shahdol, Madhya Pradesh
- Jhabua, Madya Pradesh
- Jagdalpur, Chattisgarh
- Khargone, Madya Pradesh
- Badi Sadari, Udaipur, Rajasthan
- Kanchivaram, Tamil Nadu

During the collection from a given province, cuttings 8–10 cm long and 1–1.25 cm thick were prepared and used for screening trials.

Mycorrhizal inoculum

Mycorrhizal inoculum was prepared at TERI. Four different types of AM (arbuscular mycorrhiza)

inocula were prepared through mass-produced organisms *in vitro* and used for screening trials.

The details of AM inocula used are provided on Table 1.

Table 1 AM species

Code	*AM species*
AM1	*Glomus intraradices*
AM2	*Glomus sp* 1
AM3	*Glomus sp* 2
AM4	*Glomus sp* (mixture of all three)

Screening trials for selecting mycorrhizal fungi and planting germplasm

Various mycorrhizal inoculum as indicated above were tested in fly ash on jatropha germplasm collected from various provinces to select the best combination of a AMF and plant germplasm based on better growth and hyperaccumulator properties.

The following procedure was followed while conducting the screening trials.

Assessment of infectious propagule density of mycorrhizal inocula

Prior to inoculation, to know the infectious propagule density, all the mycorrhizal inocula were subjected to bioassay test using the method of Sharma, Gaur, Bhatia, *et al.*, 1996. The inoculum density, at the rate of 2000 IP/g (infectious propagules per gram) was ascertained and further diluted with vermicompost to make it 200 IP/g substrate for inoculation.

Potting mixture, AM inoculation, and planting

The potting mixture consisted of fly ash and well rotten FYM (farmyard manure), mixed in the ratio of 4:1 w/w (weight by weight) and filled in polythene bags. The mixture was filed in black polythene bags (gussited type, 3.5" × 7"), leaving 1" space at the top to facilitate watering and other cultural operations.

Mycorrhizal inoculation was done by applying 1 g inoculum in the holes, consisting of 200 IP/ polybag or cutting at the time of planting. There were five sets of AM inoculation, including one uninoculated control for each province.

Experimental design, measurements, and statistical analysis

The treatment consisted of five types of AM inoculation (including one control that consisted the sterilized inoculum). The treatments were tested in a completely randomized design with 10 replicates. Vegetative cuttings of jatropha were grown for 16 weeks after germination. Destructive sampling for shoots and roots was done after 16 weeks of sprouting. Measurements such as heavy metal uptake and nutrient uptake were recorded after processing and analysing the samples. The biomass data (root and shoot) was recorded after drying the samples at 90 °C for 72 hours in the thermocentre. Dry weights for shoots and roots were recorded.

Phosphorus was determined using Olsen. The heavy metal and micronutrient content in plant samples was determined by drying the leaves at 90 °C for 72 hours, grinding, and digesting the samples in HF (hydrogen fluoride) on MARS5 (microwave digestion system). The digested samples were tested for various heavy metals on AAS (atomic absorption spectophotometre). The heavy metal and micronutrient concentration was expressed in PPM (parts per million). All the data was processed for statistical analysis using Co-plot software. The analysis of variance for all the data was carried out and the treatment means were separated by DMRT (Duncan's Multiple Range Test). In order to select hyper accumulating mycorrhiza and jatropha provinces, two-way factorial analysis was performed using Costat software.

Results

Biomass profile

The mycorrhizal fungi and jatropha provinces were selected in fly ash to have best hyper-AMF and plant genotypes, eventually to be established in fly ash overburdens.

The two-way analysis of biomass data presented in Table 2 revealed that irrespective of jatropha germplasm collected from various provinces, mycorrhiza inoculation produced significantly higher dry biomass (shoot + root) per plant when compared to uninoculated plants. Although there was variance in efficacy among the AM types used in the study. The plants (irrespective of province) inoculated with *Glomus* strain 1 (AM2) and mixed species of AMF (AM4) showed higher biomass when compared to other AM types used in the study.

The provincial germplasm was also found to be varied in terms of producing biomass. Irrespective of AM type inoculation, the province that performed best in terms of producing higher biomass was found to be Shahdol, Madhya Pradesh.

Overall, the main effect of AM inoculation and provinces and their interaction on biomass (shoot + root dry mass) was found to be significant.

Table 2 Biomass (dry weight of shoots + roots) of mycorrhiza mediated jatropha provinces grown in fly ash (analysed after three months of active growth)

AMF treatments	*Total dry biomass (g/plant) in various Jatropha provinces*						
	Sehore	*Khandwa*	*Shahdol*	*Jagdalpur*	*Khargone*	*Udaipur*	*Kanchivaram*
AM1	8.04	8.74	13.84	11.69	6.37	7.48	6.28
AM2	8.26	8.51	13.81	14.16	9.02	7.43	8.95
AM3	11.92	10.74	9.95	11.82	9.90	7.31	6.92
AM4	7.11	10.89	11.39	11.29	9.73	10.51	6.64
Uninoculated	6.24	7.67	10.37	7.57	7.69	6.68	6.74
Results							
2-way ANOVA							
LSD (0.05) AMF				0.72			
LSD (0.05) province				0.91			
Interaction effects							
AMF				***			
province				***			
AMF × Province				***			

ns – non significant (P= 0.05); *** – significant at 0.01

Phosphorus uptake

P (phosphorus) concentration in shoots and roots was found to influenced by AM inoculation and varied among the provinces used in the trial. The analysis of data carried out through two-way ANOVA as seen in Table 3 revealed that the mycorrhiza plants had significantly higher P content in plant tissues (irrespective of province type) when compared to un-inoculated plants. The response of various AM types used responded differently among them; the maximum P (irrespective of province type) content was recorded in plants inoculated with mixed AM (AM4) when compared to other AMF inoculation. On the other hand, the provincial response (irrespective of AM type) showed that the Shahdol, Madhya Pradesh followed by Udaipur provinces, Rajasthan were found to have higher content of P. Overall, the main effects of AM inoculation and provinces and their interaction were found to be significant.

Heavy metal profile in roots and shoots

The heavy metal data of roots and shoots was analysed by two-way ANOVA. Overall, the main effects of AMF and provinces and their interaction on heavy metal profile of a particular metal were found to be varied. The interaction was significant for aluminium, lead, and zinc, while non-significant for manganese accumulation.

Table 3 Total phosphorus uptake (roots + shoots) of mycorrhiza mediated jatropha provinces grown in fly ash (analysed after three months of active growth)

AMF treatments	*Total P uptake (ppm) in various Jatropha provinces*						
	Khandwa	*Shahdol*	*Jhabua*	*Jagdalpur*	*Khargone*	*Udaipur*	*Kanchivaram*
AM1	3547.02	4742.4	4232.98	3536.68	4140.56	6331.23	4136.01
AM2	4058.66	4793.25	4220.56	4244.35	5119.57	4790.9	2778.42
AM3	3260.5	5111.31	3416.24	4415.77	4607.81	5353.87	3646.73
AM4	4535.76	5716.78	3627.03	3799.07	4877.78	4618.93	5901.3
Uninoculated	3933.0	4115.07	3763.87	4111.16	2885.24	5505.5	4005.7
Results							
2-way ANOVA							
LSD (0.05) AMF				144.81			
LSD (0.05) province				171.34			
Interaction effects							
AMF				***			
province				***			
AMF × province				***			

ns – non significant (P = 0.05); *** – significant at 0.01; p – phosphorus; ppm – parts per million

References

Olsen S R, Cole C V, Watanabe F S, and Dean L A. 1954
Estimation of available phosphorus in soils by extraction with Sodium bicarbonate
Circular 939, US Department of Agriculture
Washington, DC: US Department of Agriculture

Sharma M P, Gaur A, Bhatia N P, and Adholeya A. 1996
Growth responses and dependence of *Acacia nilotica* var. cupriciformis on the indigenous arbuscular mycorrhizal consortium of a marginal wasteland soil
Mycorrhiza **6:** 441–446.

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.

- *Agriculture Ecosystems & Environment*
- *Agroforestry Systems*
- *American Journal of Botany*
- *Applied and Environmental Microbiology*
- *Biology and Fertility of Soils*
  Soil Biology & Biochemistry
- *Canadian Journal Of Forest Research-Revue Canadienne De Recherche Forestiere*
- *Environmental Pollution*
- *European Journal of Horticultural Science*
- *Journal of Agricultural Science*
- *Journal of Plant Nutrition*
- *Mycologia*
- *Mycological Research*
- *Mycorrhiza*
- *New Forests*
- *New Phytologist*
- *Plant and Soil*
- *Plos Biology*
- *Trends In Plant Science*

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)
Agueda B*, Parlade J, de Miguel A M, Martinez-Pena F. 2006	**Characterization and identification of field ectomycorrhizae of Boletus edulis and Cistus ladanifer** *Mycologia* **98**(1): 23–30 [*Agueda B, Consejeria Med Ambiente, Dept Invest and Experiencias Forestales Valonsadero, Junta Castilla and Leon, Apdo Correos 175, E-42080 Soria, Spain]
Ahmed F R S*, Killham K, and Alexander I. 2006	**Influences of arbuscular mycorrhizal fungus Glomus mosseae on growth and nutrition of lentil irrigated with arsenic contaminated water** *Plant and Soil* **283**(1–2): 33–41 [*Ahmed FRS, University of Aberdeen, School of Biological Science Plant and Soil Science, Cruickshank Building, Aberdeen AB24 3UU, Scotland]
Albertsen A, Ravnskov S, Green H, Jensen D F, Larsen J*. 2006	**Interactions between the external mycelium of the mycorrhizal fungus Glomus intraradices and other soil microorganisms as affected by organic matter** *Soil Biology & Biochemistry* **38**(5): 1008–1014. [*Larsen J, Res Ctr Flakkebjerg, The Danish Institute of Agricultural Sciences, Department of Integrated Pest Management, DK-4200 Slagelse, Denmark]

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos (address of the first author or of the corresponding author, marked with an asterisk)
Alkan N, Gadkar V, Yarden O, Kapulnik Y*. 2006	**Analysis of quantitative interactions between two species of arbuscular mycorrhizal fungi, Glomus mosseae and G. intraradices, by real-time PCR** *Applied and Environmental Microbiology* **72**(6): 4192–4199 [*Kapulnik Y, Agricultural Research Organization, Volcani Centre, The Department of Agronomy and Natural Resources, POB 6, IL-50250 Bet Dagan, Israel]
Alvarez M*, Gieseke A, Godoy R, Hartel S. 2006	**Surface-bound phosphatase activity in ectomycorrhizal fungi: a comparative study between a colorimetric and a microscope-based method** *Biology and Fertility of Soils* **42**(6): 561–568 [*Alvarez M, Universidad Austral de Chile, Institute of Botany, Campus Isla Teja, Casilla 567, Valdivia, Chile]
Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais J C, Roux C, Becard G, Sejalon-Delmas N*. 2006	**Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria** *Plos Biology* **4**(7): 1239–1247 [*Sejalon-Delmas N, University Toulouse 3, CNRS, UMR 5546, Pole Biotechnol Vegetales, Castanet Tolosan, France]
Blaszkowski J*, Renker C, and Buscot F. 2006	**Glomus drummondii and G-walkeri, two new species of arbuscular mycorrhizal fungi (Glomeromycota)** *Mycological Research* **110**(5): 555–566 [*Blaszkowski J, University of Agriculture, Department of Plant Pathology, Slowackiego 17, PL-71434 Szczecin, Poland]
Bougoure D S and Cairney J W G*. 2006	**Chitinolytic activities of ericoid mycorrhizal and other root-associated fungi from Epacris pulchella (Ericaceae)** *Mycological Research* **110**(3): 328–334 [*Cairney JWG, University of Western Sydney, Center for Plant and Food Science, Parramatta Campus, Locked Bag 1797, Penrith, NSW 1797, Australia]
Caglar S* and Bayram A. 2006	**Effects of vesicular-arbuscular mycorrhizal fungi on the leaf nutritional status of four grapevine rootstocks** *European Journal of Horticultural Science* **71**(3): 109–113 [*Caglar, S, Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Horticulture, TR-46100 Kahramanmaras, Turkey]
Cardoso I M* and Kuyper T W. 2006	**Mycorrhizas and tropical soil fertility** *Agriculture Ecosystems & Environment*. **116**(1–2): 72–84 [*Cardoso IM, Universida de Federal de Viçosa, Department of Soil Science, BR-3657000 Vicosa, MG, Brazil]
Cavagnaro T R*, Jackson L E, Six J, Ferris H, Goyal S, Asami D, Scow K M. 2006	**Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production** *Plant and Soil*. **282**(1–2): 209–225 [*Cavagnaro TR, University of California, Davis, Department of Land Air and Water Resources, 1 Shields Ave, Davis, CA 95616, USA]
Chalot M*, Blaudez D, and Brun A. 2006	**Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface** *Trends In Plant Science*. **11**(6): 263–266 [*Chalot M, University Nancy 1, Faculty of Science & Tech, Institut National de la Recherche Agronomique, UMR 1136 Interact Arbres Microorganismes, IFR Genom Ecophysiol and Ecol Fonctionnelles 110, BP 239, F-54506 Vandoeuvre Les Nancy, France]

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)
Chen Y L*, Kang L H, Malajczuk N, Dell B. 2006	**Selecting ectomycorrhizal fungi for inoculating plantations in south China: effect of Scleroderma on colonization and growth of exotic Eucalyptus globulus, E urophylla, Pinus elliottii, and P-radiata.** *Mycorrhiza* **16**(4): 251–259 [*Chen Y L, Murdoch University, School of Biological Science and Biotechnology, Perth, WA 6150, Australia]
Chen Y L*, Dell B, and Malajczuk N. 2006.	**Effect of Scleroderma spore density and age on mycorrhiza formation and growth of containerized Eucalyptus globulus and E-Urophylla seedlings** *New Forests* **31**(3): 453–467 [*Chen Y L, Murdoch University, School of Biological Science and Biotechnology, Perth, WA 6150, Australia]
Clemmensen K E*, Michelsen A, Jonasson S, Shaver G R. 2006	**Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems** *New Phytologist* **171**(2): 391–404 [*Clemmensen K E, University of Copenhagen, Department of Terrestrial Ecology, Institute of Biology, Oester Farimagsgade 2D, DK-1353 Copenhagen K, Denmark]
Duckett J G, Carafa A, and Ligrone R*. 2006.	**A highly differentiated glomeromycotean association with the mucilage-secreting, primitive antipodean liverwort Treubia (Treubiaceae): clues to the origins of mycorrhizas** *American Journal of Botany* **93**(6): 797–813 [*Ligrone R, University of Naples 2, Dipartimento Sci Ambientali, Via A Vivaldi 43, I-81100 Caserta, Italy]
Gagne A, Jany J L, Bousquet J, Khasa D P*. 2006	**Ectomycorrhizal fungal communities of nursery-inoculated seedlings outplanted on clear-cut sites in northern Alberta** *Canadian Journal Of Forest Research-Revue Canadienne De Recherche Forestiere* **36**(7): 1684–1694 [*Khasa D P, University of Laval, Chaire Rech Canada Genom Forestiere and Environm, Ste Foy, PQ G1K 7P4, Canada]
Gai J P, Feng G, Christie P, Li X L*. 2006	**Screening of arbuscular mycorrhizal fungi for symbiotic efficiency with sweet potato** *Journal of Plant Nutrition* **29**(6): 1085–1094 [*Li X L, China Agricultural University, College of Agricultural Resources and Environmental Sciences, Department of Plant Nutrition, 2 Yuan Ming Yuan W Road, Beijing 100094, Peoples Republic of China]
Guo T, Zhang J L*, Christie P, Li X L. 2006	**Effects of arbuscular mycorrhizal fungi and ammonium: nitrate ratios on growth and pungency of onion seedlings** *Journal of Plant Nutrition*. **29**(6): 1047–1059 [*Zhang J L, China Agricultural University, College of Resources and Environmental Sciences, Ministry of Education, Key Laboratory for Plant Soil Interaction, Beijing 100094, Peoples Republic of China]
Horton T R*. 2006	**The number of nuclei in basidiospores of 63 species of ectomycorrhizal Homobasidiomycetes** *Mycologia* **98**(2): 233–238 [*Horton T R*, SUNY College of Environmental Sciences and Forestry, Department of Environment and Forest Biology, 350 Illick Hall, Syracuse, NY 13210, USA]

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)
Jefwa J M*, Sinclair R, and Maghembe J A. 2006	**Diversity of glomale mycorrhizal fungi in maize/sesbania intercrops and maize monocrop systems in southern malawi** *Agroforestry Systems* **67**(2): 107–114 [*Jefwa J M, National Museums of Kenya, Herbarium PO 45166, Nairobi, Kenya]
Joner E J*, Leyval C, and Colpaert J V. 2006	**Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons both within and beyond the rhizosphere** *Environmental Pollution*. **142**(1): 34–38 [*Joner E J, Norwegian Centre for Soil and Environmental Research, Fredrik A Dahls Vei 20, N-1432 As, Norway]
Juniper S and Abbott L K*. 2006	**Soil salinity delays germination and limits growth of hyphae from propagules of arbuscular mycorrhizal fungi** *Mycorrhiza* **16**(5): 371–379 [*Abbott L K, University of Western Australia, Faculty of Natural and Agricultural Sciences, School of Geography and Earth Resources, Nedlands, WA 6009, Australia]
Korkama T, Pakkanen A, and Pennanen T. 2006	**Ectomycorrhizal community structure varies among Norway spruce (Picea bies) clones** *New Phytologist* **171**(4): 815–824 [*Korkama T, Finnish Forest Res Inst Metla, Vantaa Res Unit, POB 18, FI-01301 Vantaa, Finland]
Paszkowski U*, Jakovleva L, and Boller T. 2006	**Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis** *Plant Journal* **47**(2): 165–173 [*Paszkowski U, University of Geneva, Department of Plant Biology, CH-2011 Geneva, Switzerland]
Midgley D J*, Jordan L A, Saleeba J A, McGee P A. 2006	**Utilisation of carbon substrates by orchid and ericoid mycorrhizal fungi from Australian dry sclerophyll forests** *Mycorrhiza* **16**(3): 175–182 [*Midgley D J, University of Sydney, School of Biological Sciences, Macleay Building, A12, Sydney, NSW 2006, Australia]
Paszkowski U*, Jakovleva L, and Boller T. 2006	**Maize mutants affected at distinct stages of the arbuscular mycorrhizal symbiosis** *Plant Journal* **47**(2): 165–173 [*Paszkowski U, University of Geneva, Department of Plant Biology, CH-2011 Geneva, Switzerland]
Porras-Soriano A, Marcilla-Goldaracena I, Soriano-Martin M L, Porras-Piedra A*. 2006	**Development and resistance to Verticillium dahliae of olive plantlets inoculated with mycorrhizal fungi during the nursery period** *Journal of Agricultural Science* 144(2): 151–157 [*Porras-Piedra A, University of Castilla-La Mancha, Ronda de Calatrava 7, E-13071 Ciudad Real, Spain]
Rillig M C* and Mummey D L. 2006	**Mycorrhizas and soil structure** *New Phytologist* **171**(1): 41–53 [*Rillig M C, University of Montana, Microbial Ecology Program, Division of Biological Sciences, Missoula, MT 59812, USA]

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)
Scagel C F* and Schreiner R P. 2006	**Phosphorus supply alters tuber composition, flower production, and mycorrhizal responsiveness of container-grown hybrid Zantedeschia** *Plant And Soil*. 283(1–2): 323–337 [*Scagel C F, Department of Agriculture (USDA) Agricultural Research Service (ARS), Horticulture Crops Research Laboratory, 3420 NW Orchard Avenue, Corvallis, OR 97330, USA]
Schalamuk S, Velazquez S, Chidichimo H, Cabello M*. 2006	**Fungal spore diversity of arbuscular mycorrhizal fungi associated with spring wheat: effects of tillage** *Mycologia* **98**(1): 16–22 [*Cabello M, Instituto de Botánica Spegazzini, Avenue 53 477, RA-1900 La Plata, Argentina]
Wilson G W T*, Hartnett DC, and Rice C W. 2006	**Mycorrhizal-mediated phosphorus transfer between tallgrass prairie plants Sorghastrum nutans and Artemisia ludoviciana** *Functional Ecology* **20**(3): 427–435 [*Hartnett D C, Kansas State University, Division of Biology, Ackert Hall, Manhattan, KS 66506, USA]

Publication of research findings

Mycorrhiza News invites short papers on the subject for publication from mycorrhizologists. Papers may be sent to:

The Editor
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Papers sent in print form should be followed by soft copies, carrying the complete mailing address, telephone number, and fax number of the author/s. E-mails may be sent to <aloka@teri.res.in> or <tpsankar@teri.res.in>.

Forthcoming events

Conferences, congresses, seminars, symposia, and workshops

9–11 November 2006
Bangalore, **Inida**

Fifth Conference of the AFITA (Asian Federation for Information Technology in Agriculture) 2006
Dr V C Patil
Organising Secretary, AFITA 2006
Indian Society of Agricultural Information Technology (INSAIT)
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16–17 November 2006
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Chairman of Organizing committee, 2nd Biotech congress, Rajiv Gandhi Biotechnology Centre, L I T Premises, R T M Nagpur University
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2–4 February 2007
Delhi, **India**

30th All-India Cell Biology Conference and Symposium on *Molecules to compartments: cross-talks and networks*
Dr Anju Shrivastava
AICB Conference Secretariat, Department of Zoology University Delhi (North Campus), New Delhi – 110 007, India

Tel. (91) 11 27666051
E-mail 30.aicbc2007@gmail.com

29–30 March 2007
Oxford, **United Kingdom**

RNAi2007: Conference and Exhibition on *The expanding roles of small RNAs*
Dr M Sohail
Department of Biochemistry, South Parks Road, University of Oxford
Oxford, OX1 3QU, England, United Kingdom

Fax +44 (0) 1865 275259
E-mail muhammad.sohail@bioch.ox.ac.uk
Website http://www.libpubmedia.co.uk/Conferences/RNAi2007/Home.htm

15 January to
3 February 2007
New Delhi, **India**

Training workshop on Advanced Techniques in Mycorrhizal Research
Dr Reena Singh
Area Convenor and Associate Fellow, Centre for Mycorrhizal Research
T E R I, Darbari Seth Block, Lodhi Road, New Delhi – 110 003

Tel. 24682100/24682111 (Extn. 2620)
E-mail reenas@teri.res.in

Training workshop on advanced techniques in mycorrhizal research

15 January to 3 February 2007
TERI, India Habitat Centre, New Delhi

Organized by
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Department of Biotechnology
Government of India

TERI is organizing a workshop for mid-career scientists to impart them training on different aspects of the mycorrhizal fungi. Practical laboratory sessions will be supported by lectures and discussions. Technical manuals containing all experimental protocols will be provided to participants.

The course is of three-weeks duration, and a Certificate of Completion will be provided upon conclusion. Enrollment is for 15 participants.

Objectives

The main objective of the course is to impart hands-on training in research techniques on mycorrhizal research so that the participants can apply them in their research programmes. Therefore, besides demonstrating the techniques, participants should be encouraged to carry out the techniques/laboratory exercises themselves. The specific areas include basic aspects, applied aspects, advanced techniques, and promoting understanding on the relevance of mycorrhizal research in Indian context.

Participants

Mid-career scientists/technologists holding regular positions in universities/national laboratories/research institutes/in-house R&D (research and development) centres, and have been sponsored by their parent institutions, would be given preference in the selection as participants. Candidates' research experience, R&D facilities available with their institutions, and the utility of the training course in their research activities would be the main consideration for selecting participants.

Registration fee

- The registration fee is Rs 10 000 per participant (additional registration from the same organization qualifies for 10% discount).
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- The registration fee covers workshop kit (laboratory manual) and working lunch/tea.

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Editor Alok Adholeya • **Associate Editor** T P Sankar • **Assistant Editor** Jaya Kapur

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