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About TERI

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

The Bioresources and Biotechnology Division

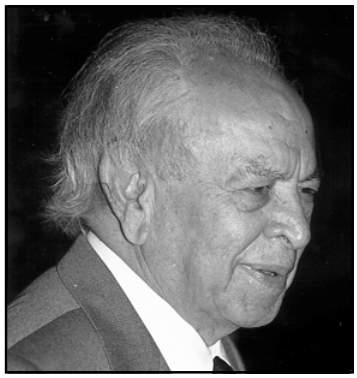
Focusing on ecological, environmental, and food security issues, the Division's activities include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grassroots level, with village communities. The Division functions through five areas – Centre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

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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



Mr Darbari S Seth
1 January 1920 – 8 December 1999

Mr Darbari S Seth was a unique individual whose vision and drive earned him the title of **'the man with the Midas touch'**. He was the builder of Tata Chemicals Limited. The Tata Chemicals plant is located in Mithapur, Gujarat – an extremely resource-poor region of the country. It was the genius of Mr Seth that conceptualized the process layout and design of this facility, using large quantities of sea water to produce chemicals that are of great value for several industrial activities in the country. Tata Chemicals also produces and supplies common salt, which is iodized by the company at no cost to the consumer and has become a widely respected household name. Mr Seth's gift benefited not only Tata Chemicals but also a number of different companies under the Tata Group. As and when these were brought under his charge at various stages, he turned them around to generate record profits and reach peak levels of performance. At some stage, he was the Chairman of 14 companies including outstanding performers like Tata Chemicals, of course, Tata Tea, Consolidated Coffee, Excel Industries, and Rallis India Limited among others.

Mr Seth's vision and gift extended far beyond the corporate sector. He pioneered and launched several initiatives of social relevance and national importance. He was always one who believed in and practised the efficient use of energy and the promotion of renewable sources for the production and supply of energy.

It was in this spirit that Mr Seth decided to establish TERI – the Tata Energy Research Institute – in the early 1970s. Always one for utilizing a share of the profits of companies that he led for larger social purposes, he provided a decent sum from two of the companies he chaired for establishing TERI's corpus. He was eager to see the development of the Institute such that it would make a difference to the way people think about energy and help create an energy structure that would minimize pollution and harmful environmental impacts. His style of functioning as Chairman of TERI was to provide encouragement, inspiration, and moral support to the Director and staff of the Institute without ever interfering in the operational decision-making.

He grew to regard TERI as the jewel in his crown, and remained Chairman of the Institute till his last day. Every little achievement of TERI provided Mr Seth with enormous joy and pride. A great source of satisfaction for him was the reality that the Institute had rapidly grown to a size and geographical spread perhaps even beyond his own expectations, and was able to generate resources and revenues to continuously expand and maintain its activities in a viable and financially autonomous manner. His advice to the Director and staff of TERI always emphasized the enormous value of financial autonomy, which he rightly believed was a prerequisite to intellectual autonomy.

TERI has grown as a family in the last 17 years that it has been functioning as a research institution. The passing away of the head of the family causes inconsolable grief to all members of this family. It is poignant that while TERI is on the eve of celebrating, in an eloquent fashion, the completion of 25 years of its formal existence, the great visionary whose gleam in the eye led to the foundation of TERI is no more. All the members of the TERI family have, on this sad occasion, resolved to dedicate all their efforts in living up to the ideals established by Mr Darbari Seth and striving to reach the goals that his aspirations defined for this Institute.

Contents

Effect of edaphic and climatic factors on the development of mycorrhiza in tree nurseries (part II): effect of soil pH, light, and carbon dioxide

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Edaphic and climatic factors such as moisture, soil texture, temperature, pH, light, and carbon dioxide may affect the efficiency of mycorrhizal fungi. This article includes the effect of soil pH, light, and carbon dioxide.

Effect of soil pH

Soil pH is a major edaphic factor which affects the establishment and efficiency of mycorrhiza in tree nurseries.

Effect of pH on ectomycorrhiza

In studies conducted at the Commonwealth Scientific and Industrial Research Organization, Division of Forestry, Wembley, Australia, seedlings of *Eucalyptus globulus* were grown in soils of pH 5 and 6 and were inoculated with one of the four fungi – *Laccaria laccata* (isolates A and B), *Pisolithus tinctorius* (isolates A and B), *Descolea maculata*, and *Setchelliogaster* sp. The plants were grown in pots containing phosphorus-deficient sand in a temperature-controlled glasshouse. The seedlings were harvested 89 days after planting. In uninoculated seedlings, less than 5% of the fine roots were infected with mycorrhiza. Inoculation with all isolates increased the phosphorus uptake at both pH levels. Growth responses were greater at pH 6 particularly for *L. laccata* isolates. Isolates which colonized roots most extensively increased plant growth to the maximum. At pH 5, *D. maculata* was the most effective fungus while at pH 6, *D. maculata* and *L. laccata* (isolate A) were most effective. *L. laccata* isolates formed more hyphae in soil (on a per pot per metre of fine root and per metre of colonized fine root basis) than other fungal isolates but were not always more effective in increasing plant growth (Thomson et al. 1996).

Studies were conducted at the Department of Chemical Ecology and Ecotoxicology, Lund University, Sweden on the growth and assimilation of ^{15}N-labelled ammonium and nitrate at pH of 4.0, 5.1, and 6.1 by *Betula pendula* and *Picea abies* seedlings. The seedlings were colonized with a common mycelium of *Paxillus involutus,* and grown together in plexiglass observation chambers. The results revealed no firm conclusions regarding the effect of pH on preferential uptake of ammonium and nitrate. However, mycelial growth of *Pisolithus tinctorius* was severely hampered at pH 6.1 (Ek et al. 1994).

The influence of mycorrhizal infection (*Suillus collinitus*) on ionic exchanges in the roots of *Pinus pinaster* was examined in studies conducted at the Laboratories de Science du Sol INRA (Institut Nationale de la Recherche Agronomique), Montpellier, Cedex, France. Rhizosphere pH and quantities of protons released by the roots of non-mycorrhizal and mycorrhizal *Pinus pinaster* were measured. The inoculation significantly enhanced the capacity of roots to release protons in a medium with neutral pH but the strength of reaction varied with the part of the root involved (Rigou et al. 1995).

Studies were conducted at the University of Gottingen, Department of Plant Physiology, Haren, The Netherlands on *Pseudotsuga menziesii* seedlings. The seedlings showed healthy growth in a medium with defined ionic ratios and pH 4. However, use of a slightly different solution, lower in phosphate, resulted in acidification of the medium (pH less than

*Compiled from TERI database – Riza

3.5) which was fatal to mycorrhizal formation (Kamminga-van and Prins 1990).

Studies were conducted on *Picea abies* and *Betula pendula* seedlings at the Lund University, Department of Microbial Ecology, Lund, Sweden. The seedlings were uninoculated or inoculated with *Paxillus involutus* and grown in unamended peat (pH 4) or in peat amended with lime (to obtain a pH of 5.1 or 6.1). The results showed that increase in pH did not affect mycorrhizal colonization. Mycorrhizal colonization increased the uptake of labelled phosphorus at pH 4 and 5.1. This was, however, greatly reduced at pH 6.1 in both mycorrhizal and non-mycorrhizal plants. Mycorrhizal colonization increased uptake of labelled calcium at pH 4 but at higher pH levels, uptake of calcium by non-mycorrhizal plants was either same or more than that in mycorrhizal plants (Andersson, Jensen, and Soderstrom 1996).

Studies were also conducted at the same university to determine the effect of pH gradients in humus on the growth and survival of seven ectomycorrhizal isolates. Humus was collected from 40-year-old forest of *Pinus sylvestris* which had been treated with wood ash (317.5 t/ha) and dolomite lime (2–5 t/ha). The seven ectomycorrhizal isolates were grown on half strength modified Melin–Norkran's medium incorporated in unsterilized humus in petriplate system. *Paxillus involutus* was the only fungus affected both by the increase in pH and by the different treatments applied in relation to growth and survival in humus, infection potential (tested by introducing four-week-old *Pinus sylvestris* seedlings into the growth system), and competitive ability. No other fungus grew saprophytically but they showed similar changes in infection potential in response to pH irrespective of whether lime or ash had been added (Erland and Soderstrom 1991).

Studies were conducted at the Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois, USA on *Quercus palustris* (pin oak) seedlings. The seedlings were grown for two years on acidic (pH 5.5) or alkaline (pH 7.5) media and inoculated with soil from three sites – a native pin oak forest site, urban site with chlorotic pin oak trees, and urban site with non-chlorotic pin oak trees. Ectomycorrhizal formation in pin oak roots was similar for soil inocula from all sites and for both pH treatments and was lacking in the control inoculated with autoclaved soil. Seedling biomass was greater in the acidic medium than in the alkaline medium. Also, inoculated seedlings in alkaline medium had greater biomass than the control seedlings. Mean iron concentration of seedlings grown in acidic medium (54.3 ppm) was significantly greater than that of seedlings grown in alkaline medium (48.7 ppm). Inoculated seedlings had similar mean leaf iron concentrations despite the differences in the pH – 49.3 ppm for acidic medium and 52.7 ppm for alkaline medium. Mean leaf iron concentration was lowest for the uninoculated seedlings in alkaline medium (44.8 ppm) (Hauer and Dawson 1996).

In studies conducted at the Universidad de Murcia, Facultad de Biologia, Department de Espinardo, Murcia, Spain, *Pinus halepensis* seedlings were grown in pots uninoculated or inoculated with *Pisolithus collinitus*. The seedlings were grown on calcareous soil or on vermiculite adjusted to pH 7.5, 6.0, 4.5, and 3.0 either with sulphuric and nitric acid (2:1 v/v) or 10% NaOH. Although no visible effects on aerial parts were observed, a reduction in root length was noted in the most acidic treatment. Enhancement of ectomycorrhizal formation was also recorded in this treatment. In substrate of neutro basic pH, short-term exposure to acid rain positively affected ectomycorrhizal fungi, in particular *Suillus* spp. (Honrubia and Diaz 1996).

Studies were conducted on *Eucalyptus urophylla* seedlings at the School of Biological and Environmental Sciences, Murdoch University, Western Australia. The seedlings were inoculated with seven *Pisolithus* isolates, *Scleroderma cepa,* and *Laccaria laccata* and transplanted in pots containing non-sterile acidic sandy loam soil (pH 4–6) amended with four levels of $CaCO_3$ which raised soil pH from 4.6 to 6.6. The plants were subjected to 28 ± 2 °C in temperature-controlled water baths. The increase in soil pH from 4.6 to 6.6 significantly decreased plant dry weight and shoot nutrient content of both uninoculated and inoculated seedlings. Four *Pisolithus* isolates (H445, H2144, M56, and H4004) significantly increased the seedling growth at pH 4.6 and eight of the ectomycorrhizal isolates (seven *Pisolithus* isolates and *L. laccata*) significantly improved total dry weight when compared with uninoculated seedlings; *Pisolithus* isolates increased the total dry weight more than *L. laccata*. *S. cepa* was ineffective at all pH levels. *Pisolithus* strain H445 increased total dry weight by 147% at 8 mg/kg soil phosphorus compared to uninoculated plants. However, the total dry weight was 70% of the plants fertilized with 64 mg/kg soil phosphorus at pH 4.6. At pH 5.8 and 6.6, M56 strain was the best growth promoter (Aggangan, Dell, and Malajczuk 1996).

Effect of pH on vesicular–arbuscular mycorrhiza

Studies were conducted at the Department of Soil Science, Oregon State University, Corvallis, Oregon, USA on sweetgum (*Liquidambar styraciflua*) seedlings. Strongly acidic surface soil (with 8.87% organic

matter) and subsurface soil (with 0.1% organic matter) were limed with increments in calcium carbonate to attain a pH range of 5.0–8.1. In the absence of VAM (vesicular–arbuscular mycorrhiza) inoculation, neither surface soil nor subsurface soil supported the growth of sweetgum seedlings at any pH despite regular (biweekly to weekly) supplements of phosphorus-shorted Long Ashton's complete nutrient solution. Two species of *Glomus* strikingly enhanced the growth of sweetgum (as measured by stem height, collar diameter, leaf number, leaf area, and tissue dry weight) but to different degrees depending on apparent pH tolerance of the VAM species. *Glomus fasciculatum* with about 40% root colonization stimulated the earliest (within four weeks) and longest growth response at pH 5.1 and 5.9. Conversely, *G. mosseae* with 15% colonization stimulated equivalent growth, although later (at about three months) but at the higher pH (6–8.1) and did not enhance growth at pH 5.1 although it colonized the roots at this pH (Davis, Young, and Linderman 1983).

To quantify the mechanisms by which acidity might affect movement and uptake of phosphorus by black locust (*Robinia pseudoacacia*), studies were conducted at the Department of Forestry and Natural Resources, Purdue University, West Lafayette, USA. There was up to fivefold increase in phosphorus diffusion rate with an increased acidity of one pH unit. In mycorrhizal (*Glomus fasciculatum*) plants, the rate of phosphorus uptake at pH 4 was twice that observed at pH 7, while in non-mycorrhizal plants, uptake rates were similar at both pH levels. The contribution of soil nutrient supply mechanisms to plant nutrient content increased with rhizosphere acidification. Uptake kinetics were also affected by rhizosphere acidification (Gillespie and Pope 1991).

Studies were conducted at the University of Missouri, Columbia, USA on *Acer saccharum* seeds which were planted in silt loam soil. Living or dead inoculum of one of the four VAM fungi (*Glomus clarum, G. epigaeum, G. macrocarpum,* and *G. mosseae*) was introduced and the soil was either not amended or amended with 1 or 2 μg H^+ per 100 g added as hydrogen chloride, 3 or 6 μg OH^- per 100 g added as calcium hydroxide, and 3 μg OH^- per 100 g added as magnesium hydroxide. Colonization with VAM fungi increased the mean leaf number per seedling from 2.8 to 3.7 and mean lateral root number from 24 to 43. *G. macrocarpum* most strongly affected seedling variables. Maximum VAM production (44% of the root length colonized) was with *G. macrocarpum* and added magnesium. Only *G. clarum* induced mycorrhizal growth at low pH but had little effect on seedling variables during the 19-week growing period. Generally, root measurements were directly related to soil pH. Maximum measurements were approximately twice those of minimum and occurred when 3 μg OH^- was added as calcium hydroxide (Reid et al. 1988).

Studies were conducted on sugar maple (*Acer saccharum*) seedlings at the Direction de la Recherche, Ministere des Resources Naturelles, Rue Einstein, Sante-Foy, Quebec, Canada. The seedlings were grown in a glasshouse in soil with five levels of soil base saturation (12–50) and either uninoculated or inoculated with endomycorrhizal fungus. After three months, the shoot elongation rate, growth (dry matter, stem diameter, and total leaf area), and potassium, calcium, and magnesium concentrations in foliage and roots decreased with soil acidification. Base saturation was used as an indicator of the status of soil acidification. Foliar potassium and calcium levels reached values less than critical thresholds when soil base saturation was reduced to 12. Seedling growth was not significantly affected by endomycorrhizal colonization, except for root dry matter which was reduced by 24% as compared to the control. For mycorrhizal seedlings, foliar aluminium concentration increased linearly (from 113 mg/kg to 210 mg/kg) with the reduction in soil base saturation (from 50 to 12) while for non-mycorrhizal seedlings, foliar aluminium concentration remained at a high level (195 mg/kg) independent of the level of soil acidification. Seedling growth was, however, not related to the accumulation of aluminium in the foliage (Ouimet, Camire, and Furlan 1996).

Relative tolerance of *Glomus mosseae*, *G. fasciculatum, and G. macrocarpum* to graded pH levels (7.8–10.5) and their influence on phosphorus uptake in *Prosopis juliflora* were evaluated in studies conducted at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. The results showed that increase in pH adversely affected growth, biomass, and phosphorus concentration in seedlings. Chlamydospore formation in the rhizosphere soil by all three VAM fungi decreased with increase in pH. However, application of VAM resulted in a significant increase in root–shoot length, collar diameter, and seedling biomass at high pH levels. The biomass of mycorrhizal plants grown at pH 10.5 was equivalent to that of uninoculated plants at pH 7.8. Root phosphorus concentration increased by 68% at pH 7.8 and 40% at pH 10.5 in VAM seedlings. *G. fasciculatum* originally isolated from site having high pH (pH 9.2) had relatively high tolerance to pH 8.5–10.5 compared to other species as exhibited by higher degree of chlamydospore formation and root colonization (Sidhu and Behl 1997).

Effect of pH and fertilizer application on mycorrhiza

Soil pH and available soil nutrients have a cumulative effect on the efficiency of VAM on tree seedlings. There is, therefore, a need to find out soil pH and minimum available soil nutrients (especially the levels of nitrogen, phosphorus, and potassium) at that pH to obtain maximum plant growth.

Effect of pH and phosphorus application on VAM efficiency

In studies conducted at the Iowa State University, Ames, USA, the response of sweetgum (*Liquidambar styraciflua*) seedlings, grown without or with *Gigaspora margarita* in soil with 25, 50, and 100 ppm soil phosphorus and adjusted to pH of 4.5, 5.5, 6.5, and 7.8 was observed during the first growing season. Best seedling growth for both VAM and non-VAM treatments occurred at soil pH 4.5 and 100 ppm soil phosphorus where mean height was 28.2 cm and top dry weight 8 g. Mean heights at pH 4.5 at 25, 50, and 100 ppm soil phosphorus were 20.1, 23.2, and 28.2 cm, respectively for VAM seedlings and 8.6, 23.5, and 31.7 cm, respectively for non-VAM seedlings. A similar relationship was observed at pH 5.5, although with smaller mean heights. As soil pH increased, seedling growth decreased significantly and at pH 7.8, the average height of the seedlings was 4 cm irrespective of the soil phosphorus concentration and mycorrhizal status. Seedling growth at all pH levels, except pH 7.8, decreased with decreasing soil phosphorus concentration. VAM seedlings were significantly larger than non-VAM seedlings at pH 4.5 and 5.5 and 25 ppm soil phosphorus (Yawnfy, Schultz, and Kormanik 1981 and 1982).

Studies were conducted at the Soil Science Department, University of Pertanian, Malaysia, Salangor, Malaysia on cocoa (*Theobroma cacao*) plants supplied with phosphorus fertilizers (0, 90, and 180 kg/ha) for pH of 5.0, 5.5, 6.0, and 6.5. The degree of plant responses – as measured by plant height, girdle diameter, leaf number, and weight of tops – varied with soil pH and the concentration of phosphorus fertilizers (Jamaluddin and Azizah 1985).

In studies conducted at the Plant Central Research Agroforestry, Nairobi, Kenya, *Leucaena leucocephala* seedlings were raised on 12 nutrient-depleted farm soils with pH range 5.6–6.7 and with or without application of rock phosphate and farmyard manure. The results showed that soil pH and root infection, 25 days after planting, accounted for much of the variation in final shoot weight among soils with no ameliorants (87%). As early root infection increased from 20% to 40% at soil pH 5.0, the predicted final shoot dry weight doubled and the response to ameliorants reduced to two-thirds. The growth responses to increased infection by VAM fungi reduced as pH increased from 5.0 to 6.5 (Shepherd et al. 1996).

Effect of pH and nitrogen application on VAM efficiency

Studies were conducted at the Department of Forestry and Wood Technology, Faculty of Agriculture, Alexandria University, Egypt on one-year-old seedlings of *Eucalyptus camaldulensis.* The seedlings were grown in sandy soil containing spores of *Acaulospora laevis*, *A. trappeis*, and *Gigaspora tricalypt* and treated with different ratios of NH_4–NO_3 (ammonium–nitrate). The stem height and fresh and dry weights of shoots and roots did not show significant difference for treatments with various NH_4–NO_3 ratios and pH range of 9.8–3.1. However, only nitrate as source of nitrogen at pH 3.1 gave the highest fresh and dry weights of mature leaves. The highest root length was obtained with NH_4–NO_3 ratio of 80:20 at pH 9.7 (Abouelkhair 1993).

Effect of pH and nitrogen application on ectomycorrhizal efficiency

Studies were conducted at the Institutes for Mycorrhizal Research and Development, United States Department of Agriculture, Forest Service, Southeastern Forest Experiment Station, Athens, USA on five families of loblolly pine (*Pinus taeda*). These were grown from April to February on fumigated soil at pH 4.8, 5.8, or 6.5 in outdoor plywood microplots. The results showed that vegetative inoculum of *Pisolithus tinctorius* buried in soil significantly lost viability after 54 days at soil pH 6.8. Inoculum viability declined in all treatments after 81 days but was only a little over one-third as viable at pH 6.8 as at lower pH levels. Up to three applications of ammonium nitrate (50 kg nitrogen per hectare each) did not affect the inoculum viability and the five loblolly pines showed similar reaction. Seedling height, root collar diameters, and top dry weights were more affected by soil pH than by nitrogen application. Seedling growth decreased with increase in soil pH. Total *P. tinctorius* ectomycorrhizal ratings (combining number of mycorrhiza and proportion of different mycorrhizal types) were about one-fourth as much at pH 6.8 as in more acidic conditions (Marx 1990).

In another study conducted at the same university, *Pinus sylvestris* seedlings were grown in plexiglass observation chambers in limed ($CaCO_3$: pH 5.0 and 5.9) and unlimed (pH 4.1) peat. These were either not colonized or colonized with *Paxillus*

involutus. After 18 weeks, ^{15}N-labelled organic nitrogen, lyophilized, and ground mycelium of *Suillus variegatus* or ammonium were added to the peat. Irrespective of the form of nitrogen added, liming decreased both the content and concentration of ^{15}N in non-mycorrhizal and, to a lesser extent, in mycorrhizal plants. In mycorrhizal plants, the uptake of ^{15}N was not correlated with the area colonized by the mycorrhizal mycelium. The amount of potassium chloride-extractable ^{15}N in peat without plants and mycorrhizal fungi decreased with liming (Andersson, Ek, and Soderstrom 1997).

Effect of pH and heavy metals on mycorrhizal efficiency

High soil pH and presence of heavy metals in soil may damage fine roots. Mycorrhizal infection on fine roots protects the roots from such damage. In studies conducted at the Institute fur Allgemaene Botanik, Johannes, Guttenberg-Universitat, Mainz, Germany, three-year-old non-mycorrhizal Norway spruce (*Picea abies*) seedlings were held for two years in spray hydroculture and then treated for six months with nutrient solution similar in composition to lysimeter solutions obtained from below spruce forests. The pH values were adjusted to 5.0, 3.5, or 2.5 and some of the nutrient solutions with pH 3.5 or 2.5 were enriched with 1 or 2 millimole aluminium per litre. The values of calcium and magnesium in needles and roots under acid and aluminium stress did not fall below the deficiency thresholds even when the contents of both elements decreased by up to 35%. Only acid stress damaged the very fine roots – lysis of peripheral root cell walls (acid holes), destruction of the calyptra, lysis of the root meristem, and, in some cases, collapse of the phloem. Aluminium intensified the damage and also caused swelling of the root tips and made the root cell walls more brittle (Vogelei and Rothe 1988).

In studies conducted at the Department of Forest Science, College of Forestry, Oregon State University, Corvallis, Oregon, USA, balsam fir (*Abies balsamea*) seedlings were inoculated with *Cenococcum geophilum*, *Hebeloma crustuliniforme*, and *Laccaria laccata*. The seedlings were grown in a substrate adjusted to pH 3, 4, and 5 and treated with aluminium (0, 50, and 100 mg/g). The seedlings were watered with one-fifth strength Arnon's solution having pH 3, 4, and 5 and containing 0, 50, or 100 mg Al per litre. As the acidity of the solution and concentration of aluminium increased, ectomycorrhizal formation, root weight, shoot weight, total weight, root–shoot ratio, and boron concentration in the seedlings decreased; however, the concentration of iron, manganese, phosphorus, and zinc increased. Concentration of nitrogen, calcium, and magnesium did not vary significantly in any of the treatments. Thus, pH and aluminium have a complex effect on ectomycorrhizal formation and nutrient uptake in *A. balsamea* seedlings (Entry et al. 1987).

Studies were conducted on *Acacia mangium* seedlings at the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, USA. The seedlings were inoculated with *Glomus aggregatum* and grown in manganese-rich oxisol at pH 4.3–6.0 and soil phosphorus concentration of 0.02 mg/litre favourable for growth of VAM plants and 0.8 mg/litre sufficient for growth of non-VAM plants. At lower phosphorus concentration, colonization of the roots increased as soil pH increased from 4.3 to 5.0 and was not significantly affected by further increase in pH. However, growth of *A. mangium* plants at lower phosphorus concentration in the presence of *G. aggregatum* was inferior to that observed at higher phosphorus concentration suggesting some degree of host–endophyte incompatibility. Increasing phosphorus concentration from 0.02 to 0.8 mg/litre increased soil pH from 4.3 to 4.8 and reduced available manganese concentration in soil from 13.2 to 2.1 mg/litre. Thus, better growth at higher phosphorus concentration at pH 5 was due to reduced manganese toxicity, probably because of precipitation of the cation directly by excess of phosphate and/or by phosphate-induced elevation of pH. Poor growth of VAM plants at low phosphorus concentration may be due to manganese toxicity (Habte and Soedarjo 1996).

Effect of light

Light is essential for the development of mycorrhiza on plant roots as increased light results in more photosynthesis and greater supply of carbohydrates to roots, thus increasing the susceptibility of roots to attack by mycorrhizal fungi. Development of mycorrhizae on roots in turn increases the rate of photosynthesis.

Effect of light on mycorrhiza and plant growth

Studies conducted at the Department of Forest and Wood Sciences, Colorado State University, Fort Collins, USA on *Pinus contorta* plants showed that increased irradiance resulted in greater mycorrhizal formation (Reid, Kidd, and Ekwebelam 1983).

In studies conducted at the Forest Research Institute, Nigeria, Ibadan, Nigeria, lodgepole pine (*Pinus contorta*) seedlings were grown for 10 weeks without ectomycorrhizae in a greenhouse at three levels of irradiance (low, medium, and high) and

three levels of ammonium nitrate (3, 62, and 248 ppm nitrogen). At 10 weeks, inoculation with either *Pisolithus tinctorius* or *Suillus granulatus* was superimposed on light and nitrogen treatments and the seedlings were grown for further six weeks. Mycorrhizal formation increased with increase in irradiance while nitrogen fertilization at 62 ppm level resulted in greater mycorrhiza formation than 3 and 248 ppm levels. Inoculation with *P. tinctorius* and *S. granulatus* resulted in photosynthetic rates of 1.87 and 1.85 mg carbon dioxide.cntdot.dm^{-2}.cntdot.h^{-1}, respectively as compared to 1.41 mg carbon dioxide.cntdot.dm^{-2}.cntdot.h^{-1} in non-mycorrhizal plants. Also, inoculated plants had significantly greater biomass and took up more phosphorus than uninoculated plants. Although increase in growth of mycorrhizal seedlings was associated with increased photosynthesis, the magnitude of this response depended on specific combination of irradiance and nitrogen fertilization (Ekwebelam and Reid 1983).

Studies were conducted at the Faculty of Forestry, Gadjah Mada University, Yogyakarta, Indonesia on *Shorea academia* seedlings. The seedlings were grown on two types of soil for three light intensities – heavy shading (range 1350–7200 lux), medium shading (range 3900–18 900 lux), and open site (19 200–87 000 lux) – and were either inoculated or uninoculated with mycorrhiza two months after germination. The results showed that light intensity significantly affected the height, diameter, biomass, survival, and mycorrhizal formation. Soil type plus inoculation did not affect these parameters. However, light intensity plus soil type, light intensity plus inoculation, and light intensity plus inoculation plus soil type significantly affected the growth parameters (Suhardi and Darmawan 1990).

In studies conducted at the Department of Botany, University of New England, Armidale, Australia, coachwood (*Ceratopetalum apetalum*) seedlings were grown with or without *Glomus mosseae*, *Trifolium pratense*, and phosphate and under shade or full sunlight. The results showed that weights of mycorrhizal seedlings were less than non-mycorrhizal seedlings grown in full sunlight. Mycorrhizal seedlings grown in the shade were heavier when grown with the companion plants than when grown without them. There was no correlation between seedling size and phosphate nutrition (McGee 1990).

Effect of mycorrhiza on photosynthesis

In studies conducted at the Colorado State University, Fort Collins, Colorado, USA, *Pinus taeda* seedlings were grown on peat vermiculite and inoculated with *Pisolithus tinctorius*. The results showed that fungal infection increased the net photosynthetic rate to more than that in the non-infected controls. Also, respiration of mycorrhizal roots was the greatest drain on current photosynthesis (Kidd and Reid 1981).

Further studies conducted at the same university on plants of *Pinus contorata* showed that at the age of 16 weeks, six weeks following inoculation with either *Pisolithus tinctorius* or *Suillus granulatus*, net photosynthetic rate and biomass were significantly greater in mycorrhizal than in non-mycorrhizal plants. Similarly, in studies on *P. taeda* plants, net photosynthetic rate of mycorrhizal plants was consistently greater than that of non-mycorrhizal plants during a 10-month period and at 10 months it was greater by 2.1-fold (Reid, Kidd, and Ekwebelam 1983).

Studies conducted at the Department of Soil Science, Oregon State University, Corvallis, Oregon, USA on Douglas fir (*Pseudotsuga menziesii*) seedlings, uninoculated or inoculated with three ectomycorrhizal fungi, showed that *Rhizopogon vinicolor* caused a significant increase in the net photosynthetic rate compared to that of non-mycorrhizal plants. *Hebeloma crustuliniforme* and *Laccaria laccata* did not affect the net photosynthetic rate. Colonization by *R. vinicolor* and *H. crustuliniforme* increased the osmotic potential in the leaf symplast compared to that of the controls while *L. laccata* did not show any such effect. In the root tips, colonization levels of *R. vinicolor*, *H. crustuliniforme,* and *L. laccata* were 36%, 93%, and 73%, respectively. While *R. vinicolor* and *H. crustuliniforme* seedlings were smaller than those of the control, *L. laccata* did not affect the size of the seedling. Only smaller *H. crustuliniforme* seedlings exhibited elevated concentration of nitrogen, phosphorus, potassium, and calcium as compared to that in non-mycorrhizal seedlings. These data demonstrate a non-nutritional basis for increased rate of photosynthesis caused by some ectomycorrhizal fungi. This can be explained by the increased photosynthate sink generated by extensive fungal growth associated with mycorrhizas (Dosskey, Linderman, and Boersma 1990).

In studies conducted at the Department of Forestry, University of Florida, Gainsville, Florida, USA, 10-week-old *Pinus taeda* seedlings were either fertilized with phosphorus (0.1, 1.0, 4.0, and 8.0 mg/g) or treated with three different concentrations of mycelial slurry of *Pisolithus tinctorius* along with 0.1 mg/g of phosphorus. After seven days, both phosphorus fertilization and mycorrhizal development caused a substantial increase in the net rate of photosynthesis and dry matter accumulation. However, neither treatment resulted in significant changes in shoot–root allocation of dry matter or shoot dark-respiration. At low and

medium levels of mycorrhizal development, the net photosynthetic response to mycorrhizal formation appeared to be due to enhanced phosphorus nutrition. However, at high level of mycorrhizal development, as much as 17% of the total increase in the net photosynthetic rate that resulted from mycorrhizal development may be attributed to mechanisms other than enhanced phosphorus nutrition (Rousseau and Reid 1990).

To monitor the physiological condition of one-year-old Douglas fir (*Pseudotsuga menziesii*) seedlings, studies were conducted at the INRA, Nancy, Laboratoire de Bioclimatologie et d, Ecophysiologie Forestieres, Champenoux, France. The seedlings were inoculated with *Laccaria laccata* or *Thelephora terrestris* and grown at phosphorus concentrations of 10 and 40 mg/litre. On the basis of measurements taken before and after transplanting in the nursery, it was found that *L. laccata* was more efficient than *T. terrestris* in increasing water use efficiency and photosynthesis, partly due to increased phosphorus nutrition. Also, phosphorus deficiency reduced both the water use efficiency and photosynthesis. Reduction in photosynthesis following transplantation was due to a nonstomatal mechanism and recovery of photosynthesis was related to regrowth of external mycelia of mycorrhizas (Guehl and Garbaye 1990).

Effect of carbon dioxide

Increase in atmospheric CO_2 (carbon dioxide) leads to increase in photosynthetic activities, which in turn favour mycorrhizal establishment on plant roots. Mycorrhization of roots may increase the assimilation of CO_2 by plants.

Effect of mycorrhiza on carbon dioxide assimilation

The partitioning of current photosynthate by pulse labelling with $^{14}CO_2$ at the end of six time intervals was examined in *Pinus taeda* in studies conducted at the Department of Forest and Wood Sciences, Colorado State University, Fort Collins, USA. The results showed that mycorrhizal plants assimilated more $^{14}CO_2$, allocated more assimilated ^{14}C to the root system, and lost a greater percentage of ^{14}C by root respiration as compared to non-mycorrhizal plants. At 10 months, the quantity of $^{14}CO_2$ respired by the roots of mycorrhizal plants per unit root weight was 3.6-fold of non-mycorrhizal plants. Although, the stimulation of photosynthesis and translocation of current photosynthate to the root system by mycorrhizal formation was consistent with the source–sink concept of sink demand, foliar nitrogen and phosphorus concentrations were also greater in mycorrhizal plants (Reid, Kidd, and Ekwebelam 1983).

In studies conducted at the Department of Forest Science, Oregon State University, Corvallis, Oregon, USA, *Pinus ponderosa* seedlings were grown in observation containers and inoculated with *Hebeloma crustuliniforme* or *Laccaria bicolor* or left uninoculated. Root tips (mycorrhizal from inoculated plants and non-mycorrhizal from the control plants) were traced on acetate sheets. The colour of each root tip was classified as light, intermediate, or dark. Roots initiated between the eighth and ninth months were monitored for the next 90 days. All root tips progressed from light brown to dark brown to black for all three treatments. *H. crustuliniforme,* and to a lesser extent *L. bicolor,* retarded this progression as compared to the controls. At the end of 12 months, seedlings were labelled with $^{14}CO_2$. The amount of ^{14}C in light *L. bicolor* and *H. crustuliniforme* was 2.3 and 1.8 times greater, respectively, than that in light control root tips. The amount of ^{14}C in mycorrhizas of inoculated seedlings and, to a lesser extent, in roots of control seedlings, decreased as they progressed from light to dark colour. *P. ponderosa* seedlings thus continue to allocate photosynthate to morphologically older roots perhaps to meet maintenance requirements or to supply carbon for growth and metabolism of extramatrical hyphae. Such allocation may enhance root longevity which would have an important influence on tree, forest, and soil carbon budgets (Durall et al. 1994).

Studies were conducted at the University of Florida, Citrus Research and Education Centre, Lake Alfred, USA on five citrus rootstocks either uninoculated or inoculated with *Glomus intraradices* and not fertilized or fertilized with phosphorus. The results showed that rootstocks with lower mycorrhizal dependency generally had greater hydraulic conductivity, transpiration, and CO_2 assimilation rates (Graham and Syvertsen 1985).

Further studies were conducted at the same university on sour orange plants raised in low phosphorus sandy soil and either uninoculated and fertilized with additional phosphorus or inoculated with *Glomus intraradices*. The results showed that nutrient status and net gas exchange varied with leaf age (expanding, recently expanded, and mature leaves). VAM fungus infected 79% of the root length and mycorrhizal plants were comparable in relative growth rate and size of final harvest with non-mycorrhizal plants. Except nitrogen, leaf mineral concentrations were generally the same for VAM and non-VAM plants. Although leaf nitrogen was apparently sufficient for high rates of net CO_2 assimilation, VAM plants had higher nitrogen concentrations than non-VAM plants at the time of gas exchange measurement. *G. intraradices* had no effect on net CO_2 assimilation, stomatal conductance, or water use efficiency irrespective of the leaf age (Syvertsen and Graham 1990).

Effect of elevated carbon dioxide on mycorrhiza

Forest tree biomass is hypothesized to increase in a CO_2-enriched atmosphere if mechanisms exist to ensure acquisition of limiting nutrients in forest soils. Investment of additional photosynthate produced at elevated CO_2 levels in mycorrhizal proliferation and root growth may provide one such mechanism. This hypothesis was tested in studies conducted at the Environmental Science Division, Oak Ridge National Laboratory, Oak Ridge, USA. Mycorrhizal density and seedling biomass were measured in shortleaf pine (*Pinus echinata*) and white oak (*Quercus alba*) grown in unfertilized forest soil in controlled environment chambers at CO_2 levels of 360 and 700 microlitre/litre. Mycorrhizal density was greater at elevated CO_2 level in both species after six weeks of exposure; in white oak, the increased density persisted for 24 weeks. Root dry weight increased by 76% in *P. echinata* and 91% in *Q. alba* and total seedling dry weight increased by 66% and 56%, respectively at CO_2 level of 700 microlitre/litre. It is thus hypothesized that increased photosynthesis at elevated CO_2 levels offsets the carbon requirement for mycorrhizal establishment on shortleaf pine. Greater mycorrhizal density and enhanced first-year root growth in both species may facilitate future nutrient acquisition supporting further biomass increase in an enhanced CO_2 atmosphere (O'Neill, Luxmoore, and Norby 1987).

In studies conducted at the Department of Environmental and Resource Sciences, University of Nevada, Reno, Nevada, USA, *Pinus ponderosa* seedlings were grown from seed in atmosphere with 700 microlitre/litre, 525 microlitre/litre, and ambient CO_2 concentrations and in a potting mixture with 68, 43, or 18 µg/g soil phosphorus. All the plants were inoculated with *Pisolithus tinctorius* shortly after emergence. After four months, shoot volume, root dry weight, and total root length of seedlings grown in 700 microlitre/litre CO_2 concentration was found to be greater than those of seedlings grown in other atmospheres regardless of phosphorus treatment. Shoot–root ratios decreased as the CO_2 concentration increased in each phosphorus treatment. After eight months, the smallest shoot volumes and root weights and lengths for each phosphorus treatment were similar to those of seedlings grown in ambient CO_2 concentration. Root weight and total root length increased as CO_2 concentration increased in soil with high phosphorus concentration but the maximum root weights and lengths in the medium and low phosphorus treatments were those of seedlings reared in 525 microlitre/litre CO_2 concentration. Nevertheless, shoot–root ratios decreased with increasing CO_2 concentration in both high and medium soil phosphorus at the second harvest and the highest shoot–root ratios in low phosphorus were that of seedlings grown in ambient CO_2 concentration. After one year, the largest shoot and root volumes in the high and medium phosphorus treatments were those of seedlings grown in 525 microlitre/litre intermediate CO_2 concentration while the reverse was true in low phosphorus treatment. The effects of CO_2 concentration in the final harvest was not significant. As is true with seedling growth, effects of CO_2 on ectomycorrhizal colonization varied temporally as mycorrhizal development was affected by the CO_2 concentration after four months; while seedlings grown in ambient CO_2 concentration exhibited the maximum percentage infection for each phosphorus treatment at second harvest, those grown in 700 microlitre/litre CO_2 concentration had the highest percentage infection after one year (Walker et al. 1995).

The importance of phosphorus and carbohydrate concentrations in influencing photosynthetic capacity of tropical forest tree seedlings under elevated CO_2 concentrations were investigated in studies conducted at the Smithsonian Tropical Research Institute, Balboa, Panama. *Bellschmiedia pendula* seedlings were grown under elevated CO_2 concentrations with or without VAM. VAM increased phosphorus concentrations in leaves, stems, and roots. It was found that maximum rates of photosynthesis under saturating levels of CO_2 and light were correlated with leaf phosphorus concentrations. VAM increased leaf carbohydrate concentrations particularly under elevated CO_2 concentrations but these levels were low and within the range observed in naturally occurring forest trees. Root carbohydrate concentrations were reduced in VAM plants as compared to non-mycorrhizal plants (Lovelock et al. 1997).

In studies conducted at the Harvard University, Department of Organism and Evolutionary Biology, Biology Laboratory, Cambridge, USA, *Betula papyrifera* saplings were grown at an ambient or elevated (700 ppm) atmospheric CO_2 concentration for 24 weeks. Elevated CO_2 concentration resulted in significant changes in the composition of the ectomycorrhizal assemblage towards morphotypes with a higher incidence of emanating hyphae and rhizomorphs (Godbold and Berntson 1997).

Studies were conducted at the University of Illinois, Department of Science Biology, Chicago, USA on hybrid poplar saplings (*Populus* x *Euramericana*) grown for five months in open bottom root boxes under high or low soil nitrogen and ambient (34 Pa) or elevated (69 Pa) CO_2 concentration. The results showed

that only in soils with high nitrogen concentration, arbuscular mycorrhizal root mass was twice as much and extramatrical hyphae were 11% longer in elevated than in ambient CO_2 concentration. Rhizosphere microbial biomass carbon was 1.7 times greater in soil with high than in soil with low nitrogen concentration and did not respond to elevated CO_2 concentration (Lussenhop et al. 1998).

In studies conducted at the Auburn University, School Forestry, White Smith Hall, USA, longleaf pine (*Pinus palustris*) seedlings were exposed to two concentrations of atmospheric CO_2 (365 or 720 µmol/mol) and two levels of soil nitrogen (0.02 or 0.20 mg/g/year) within an open top chamber. The seedlings were adequately watered for 19 weeks and then subjected to two water stress treatments (target value –0.5 or –1.5 MPa xylem pressure potential). The percentage of ectomycorrhizal short roots and number of ectomycorrhizas per unit root length were higher for seedlings grown in elevated CO_2 concentration, low nitrogen concentration, and adequate water. Interaction among main treatment variables demonstrated higher percentages of ectomycorrhizal short roots, fine root length per seedling, and total number of mycorrhizas per seedling for plants grown in high CO_2 concentration (compared with ambient) or adequate water (compared with water stress) or under high nitrogen concentration (Runion et al. 1997).

In studies conducted at the US Forest Service, Northeast Experimental Station, Delaware, USA, *Pinus rigida* seedlings were inoculated with *Pisolithus tinctorius* and grown on sand irrigated with nutrient solutions (pH 3.8) containing 0, 6.25, 12.5, or 25 mg/litre aluminium (0, 232, 463, or 927 µM Al) in growth chambers fumigated with 350 (ambient) or 700 (elevated) microlitre/litre CO_2. In the absence of aluminium at ambient CO_2 concentration, the mean total dry weights of seedlings at the higher nutrient level (0.2 strength Clark solution) were 164% greater than those at the low nutrient level (0.1 strength Clark solution). Total dry weight at elevated CO_2 concentration in the absence of aluminium was significantly greater than that in the ambient CO_2 concentration – at 0.1 strength Clark solution by 34% and at 0.2 strength Clark solution by 16%. Although visible symptoms of aluminium toxicity in roots and needles were reduced by CO_2 enrichment, there was no significant CO_2–aluminium interaction for shoot or root dry weight. The percentage of seedling roots that became mycorrhizal was negatively related to the nutrient level and was greater at elevated than at ambient CO_2 levels. Generally, elevated CO_2 concentration had little effect on the concentration of mineral nutrients in roots and needles (Schier and McQuattie 1998).

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Research findings

Endosymbiotic associations in the epiphytic orchid *Acambe praemorsa*

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Introduction

Epiphytic vascular plants contribute considerably to species richness and mineral nutrients in tropical and subtropical forest ecosystems (Benzing 1990). It is well known that mycorrhizal fungi influence the uptake of nutrients by the host. Observations by Bermudes and Benzing (1989) suggest that mycorrhizal fungi do not colonize epiphytes. The widespread occurrence of infection has led to the general assumption that mycorrhizae have an

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important role to play in epiphytic orchids also but this has not been investigated in the Indian epiphytic orchids. The present study reports the mycorrhizal status and the occurrence of yeast on the lysin pelotons of the roots of *Acambe praemorsa*.

Material and methods

Acambe praemorsa (epiphytic orchid) was collected from the Kolli hills (1500 metres above sea level) in Salem district, Tamil Nadu. The orchid was rinsed with running water and fixed in FAA (formalin-aceto-alcohol) for microscopic observations. Thin freehand sections were stained using toluidine blue O (Krishnamurthy 1988) and selected sections were photographed. Infection density was calculated as per the formula of Hadley and Williamson (1972).

Results and discussion

A cross section of the orchid root shows that fungal hyphae form peloton in cortical cells as described by Peterson and Farquhar (1994). The fungal mycelium grows intracellularly towards the interior layers of the cortex (Figure 1). Colonization occurred first in the cells of the outer layers of cortex and subsequently in the deeper layers of the cortex. These coils get digested by the host cell and serve as nutrients for the orchid (Peterson and Currah 1990) (Figure 2). The fungus belongs to the genus *Thanatephorus* (Currah 1987), and the infection density was estimated to be 31.2%.

A number of substances including sugars and phosphates are translocated by endophytic fungi to the orchid (Richardson, Peterson, and Currah 1992). One striking event is the occurrence of yeast cells on the digesting peloton of the cortical cells. In nature, yeasts are found in all habitats where sugar-rich liquid extracts or secretions are available, and have been reported mostly in the nectar of flowers, on fruits, and leaves (Schlegel 1995). This type of occurrence of yeast is already stated on the living partner. However, this is the first time that yeast has been observed in the lysin pelotons of orchid mycorrhiza probably for the utilization of sugars.

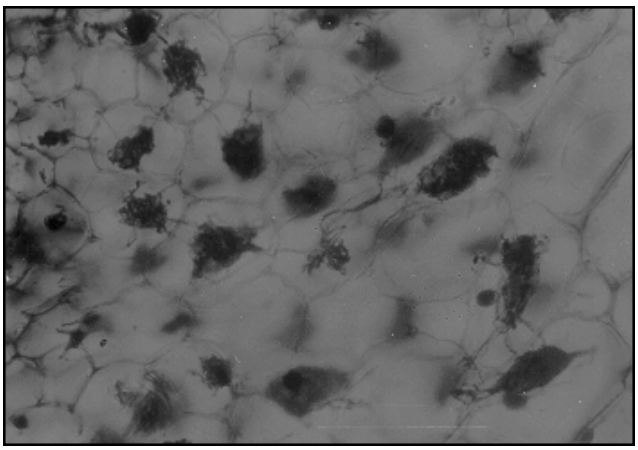

Figure 1 Cross sections of root stained with TBO show fungal pelotons (x150)

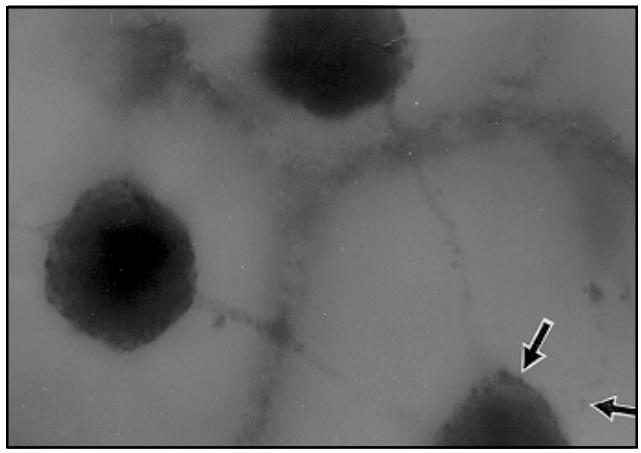

Figure 2 Fungal hyphae are seen with lysing pelotons (arrows) indicating the presence of yeast cells (x650)

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Effect of phosphorus levels on the mycorrhizal colonization, growth, yield, and nutrient uptake of cassava (*Manihot esculenta* Crantz) in alluvial soils of coastal Tamil Nadu

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Introduction

Tuber crops are now being widely recognized as one of the major sources of food in tropical countries. Cassava (*Manihot esculenta* Crantz), one of the major tuber crops, is known to have VAM (vesicular–arbuscular mycorrhizal) association on roots. Inoculation of VAM increased the tuber size and yield in cassava, elephant foot yam, and taro (Potty 1990; Ganesan and Mahadevan 1994). The present study was undertaken to see the effect of levels of phosphorus on mycorrhizal colonization, nutrient uptake, and cassava tuber yield in the alluvial soils of coastal Tamil Nadu.

Materials and methods

The culture of VAM fungus, *Glomus fasciculatum,* was maintained and mass multiplied in pots using onion as host plant. Field trial was conducted in Arangalur, Salem district, Tamil Nadu to evaluate the effect of inoculation of *G. fasciculatum* on the growth and yield of cassava as influenced by different levels of phosphorus. Three replicates were kept in the experiment and five plants were used in each replicate.

Cassava plants from each treatment were collected randomly after the ninth month and their root colonization; number of leaves; shoot length; shoot dry weight; phosphorus, potassium, and chlorophyll content; and tuber yield were recorded. The root dry weight was estimated after the fourth month. The phosphorus content was estimated using the vandomolybdate yellow colour method (Jackson 1971) and potassium content using flame photometer (Piper 1950). Chlorophyll was extracted using acetone and its absorbance was read using a spectrophotometer at 645 ηm and 663 ηm against (80% acetone) blank solvent (Aron 1949). Tryphan blue technique was adopted to detect and evaluate the VAM root colonization (Phillips and Hayman 1970).

Results and discussion

The study revealed increased plant growth for all phosphorus levels. Although the shoot length and number of leaves were highest for 100 kg P_2O_5 per hectare with VAM; the values recorded for 100 kg, 75 kg, and 50 kg P_2O_5 per hectare with VAM (and 25 kg P_2O_5 per hectare with VAM for number of leaves only), and 100 kg P_2O_5 per hectare without VAM are similar (Table 1). Sieverding and Toro (1986) have reported an increase in the root dry matter of cassava due to VAM fungal inoculation. In this study, the dry matter production observed at 50 kg P_2O_5 per hectare with VAM was similar to that from 75 kg P_2O_5 per hectare without VAM (Table 1).

Efficient nutrient uptake, especially that of phosphorus, by VAM fungus resulted in the increased root length and hence root dry matter (Table 2). In a study by Azcon, Rubio, and Barea (1991), VAM inoculation increased the concentration and content of nitrogen in plant shoot as well. An increased nitrogen content of 297.20 mg/plant was observed in 100 kg P_2O_5 per hectare with VAM, followed by 296.22 mg/plant in 75 kg P_2O_5 per hectare with VAM. In a study by George et al. (1992), external hyphae of the VAM fungi enhanced the uptake and transport of nitrogen up to 24% of the total plant uptake. In a study by Potty (1990), mycorrhizal cassava plants showed higher phosphorus uptake at low phosphorus levels. The phosphorus content of cassava for 25, 50, 75, and 100 kg P_2O_5 per hectare with VAM were 36.36, 39.07, 45.64, and 49.24 mg/plant, respectively. The control treatment (without VAM and phosphorus) has the least phosphorus content of 29.49 mg/plant. However, the phosphorus content of cassava recorded for 100 kg P_2O_5 per hectare with and without VAM was similar to 75 kg P_2O_5 per hectare with VAM.

Table 1 Effect of VAM inoculation (*Glomus fasciculatum*) and phosphorus concentration on the growth of cassava

Treatment	*Root colonization with VAM (%)*	*Number of leaves/ plant*	*Shoot length (cm)*	*Shoot dry weight (g/plant)*	*Root dry weight after fourth month (g/plant)*
Control (without VAM and phosphorus)	36.4	69.2	187.4	62.1	5.6
G. fasciculatum	72.7	70.3	202.1	64.4	6.7
25 kg P_2O_5/ha	40.5	71.9	205.6	65.9	7.3
25 kg P_2O_5/ha + VAM	80.8	75.2	208.4	68.3	8.2
50 kg P_2O_5/ha	44.6	72.2	209.8	67.8	9.1
50 kg P_2O_5/ha + VAM	82.4	74.8	220.9	73.4	10.8
75 kg P_2O_5/ha	38.4	77.6	221.1	71.2	10.4
75 kg P_2O_5/ha + VAM	75.2	81.3	230.4	84.3	13.8
100 kg P_2O_5/ha	33.5	79.9	224.1	75.5	11.8
100 kg P_2O_5/ha + VAM	69.2	87.4	230.7	84.6	14.3
SE[a]	0.6083	0.6085	0.1050	0.6085	0.4296
CD[b] (p = 0.05)	1.7291	1.7296	0.3003	1.7296	0.6075

[a] SE – standard error; [b] CD – critical difference

Table 2 Effect of VAM inoculation (*Glomus fasciculatum*) and phosphorus concentration on the nutrient content and yield of cassava

Treatment	*Nitrogen content (mg/plant)*	*Phosphorus content (mg/plant)*	*Potassium content (mg/plant)*	*Total chlorophyll content (mg/plant)*	*Tuber yield (t/ha)*
Control (without VAM and phosphorus)	267.56	29.49	174.33	1.2105	14.327
G. fasciculatum	271.23	33.42	184.64	1.2207	18.834
25 kg P_2O_5/ha	273.04	34.74	194.77	1.2316	22.115
25 kg P_2O_5/ha + VAM	277.72	36.36	210.64	1.2397	24.334
50 kg P_2O_5/ha	279.21	37.47	214.78	1.2467	26.117
50 kg P_2O_5/ha + VAM	284.32	39.07	234.33	1.2516	29.637
75 kg P_2O_5/ha	287.44	38.43	231.56	1.2597	31.056
75 kg P_2O_5/ha + VAM	296.22	45.64	274.59	1.2629	34.626
100 kg P_2O_5/ha	295.77	48.37	268.43	1.2627	34.417
100 kg P_2O_5/ha + VAM	297.20	49.24	274.94	1.2636	35.117
SE[a]	0.6906	0.5544	0.5547	0.0004	0.5837
CD[b] (p = 0.05)	1.9632	1.5760	1.5768	0.0010	1.6591

[a] SE – standard error; [b] CD – critical difference

The potassium content of cassava recorded at 100 kg P_2O_5 per hectare with VAM was similar to that for 75 kg P_2O_5 per hectare with VAM. The higher potassium uptake may be due to contact exchange theory. Highest chlorophyll content (1.2636 mg/plant) was observed in the treatment having 100 kg P_2O_5 per hectare with VAM; this was about 4% more than that for the control treatment (without VAM and phosphorus). In a study by Allen et al. (1981), mycorrhizal infection by *G. fasciculatum* increased the chlorophyll concentration by 28% in *Bouteloua gracilis*. The highest fresh weight of tuber (35.117 tonnes per hectare) was obtained at 100 kg P_2O_5 per hectare with VAM followed by 75 kg P_2O_5 per hectare with VAM (34.626 tonnes per hectare). The tuber yields obtained in 75 kg P_2O_5 per hectare with VAM and 100 kg P_2O_5 per hectare without VAM were similar. The increased root colonization by VAM might have augmented the uptake of nitrogen, potassium, phosphorus, and other minor nutrients resulting in an increase in the yield.

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Influence of *Glomus fasciculatum* inoculation on growth and phosphorus uptake in *Gladiolus* sp.

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Vesicular–arbuscular mycorrhizal association is geographically ubiquitous throughout the plant kingdom, and occurs over a broad ecological range (Mosse, Stribley, and Lectacon 1981; Gerdeman 1968). The beneficial effects of VAM (vesicular–arbuscular mycorrhizal) association are attributed to the growth-promoting aspects in many situations (particularly in infertile soils), where mycorrhizal plants grow better than non-mycorrhizal plants (Gerdeman 1968; Mosse 1973). This improved plant growth is attributed primarily to nutrient acquisition (particularly phosphorus) from soil (Gerdeman 1968), water uptake (Mosse 1981), growth-promoting substances, and biological control of soil-borne pathogens (Jalali and Chand 1988).

In the present investigation, the effect of the most common indigenous VAM fungus, *Glomus fasciculatum*, on an exotic ornamental plant, *Gladiolus* sp., is experimented.

Materials and methods

A pot-culture experiment was set up with six pots, each having 1 kg of autoclaved local soil (loamy sand with high pH and low phosphorus). Three of the pots were treated as control and in the other three, the top few layers of sterilized soil were removed and the VAM inoculum was spread on which bulbs of *Gladiolus* sp. were placed (three per pot) and covered by previously removed sand. Similarly, bulbs of *Gladiolus* sp. were sown in the pots used as control, but without adding the inoculum.

The MEI (mycorrhizal efficiency index) was calculated taking the total dry weight of the plant and using the formula of Singh and Tilak (1990).

$$\text{MEI} = 100 \times \frac{1 - \text{non-mycorrhizal plant weight}}{\text{mycorrhizal plant weight}}$$

Estimation of fresh weight and dry weight

After 60 days of growth, the plants from both the sets (control and treated) were uprooted without damaging the roots. The roots were washed in running water till the adhering soil particles were removed. The fresh weights of the root and shoot were recorded after

removing the external moisture for both control and inoculated, separately. Then the roots and shoots were oven-dried for 72 hours at 70 °C. The dry weights of root and shoot were separately recorded.

Estimation of phosphorus

Phosphorus estimation of both shoot and root of *Gladiolus* sp. was done by Vanadate method (Sekine 1965). The plant material (0.5 g) was acid digested with 20 ml concentrated nitric acid, heated, then 15 ml perchloric acid was added and the solution was condensed to 1–2 ml. Upon cooling, 50 ml of distilled water was added and filtered. The filtrate (digested extract) was used for assessment.

Results and discussion

The results are presented in Table 1. *Gladiolus* plants inoculated with the VAM fungus, *Glomus fasciculatum*, showed enhanced growth as compared to the controls. The proliferation of fibrous roots in inoculated plants was also considerably high.

The increase in fresh weight and dry weight of both root and shoot systems at the age of 60 days due to inoculation of VAM fungus was found to be significantly high (significant at five per cent level). The fresh weight of the shoot increased by 76.5% and that of the root by 50%. Similarly, as in fresh weights, the dry weight of the shoot was increased by 16.7% and that of the root by 300%.

The results presented in Table 1 show that VAM inoculation had profound effect on the uptake of phosphorus by *Gladiolus* plants. It was significantly high (significant at five per cent level) in both shoot and root due to VAM fungus inoculation. The MEI of the VAM fungus *G. fasciculatum* in enhancing the growth and phosphorus nutrition of *Gladiolus* in the local soil was found to be 17.5%.

Table 1 Fresh and dry weights and phosphorus content of the shoot and root of *Gladiolus* sp. as influenced by inoculation with *Glomus fasciculatum*

Treatment	*Fresh weight (g/plant)*		*Dry weight (g/plant)*		*Phosphorus content (mg/plant)*	
	Shoot	*Root*	*Shoot*	*Root*	*Shoot*	*Root*
Inoculated	150.0a	15.0a	35.0a	2.00a	157.0a	100.00a
Control	85.0a	10.0a	30.0a	0.50a	50.0a	75.01a

Note

In each column, values with the same letter do not differ significantly (p = 0.05)

Mycorrhizal efficiency index = 17.5%

Previous studies have indicated that phosphate deficiency is one of the important factors that limit plant growth in calcareous soils (Park, Rawes, and Allen 1962). The present native soil is calcareous with high pH (8.5); it might be true that the soils do not support good plant growth unless the plant develops symbiotic association with VAM fungus. In the present study, the inoculation of *Gladiolus* with an indigenous isolate *G. fasciculatum* in sterile soil showed a positive response with respect to growth performance. Hence from the present investigation it is clear that inoculation with *G. fasciculatum* could be highly beneficial to agriculture.

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Centre for Mycorrhizal Culture Collection (CMCC)

List of cultures available from CMCC as on December 1999

S No.	Bank code	Name of the fungus	Host
1	EM-1197	*Lactarius controversus*	*Populus euramericana*
2	EM-1202	*Lactarius rufus*	—
3	EM-1204	*Lactarius subdulcis*	*Chene*
4	EM-1266	*Paxillus involutus*	—
5	EM-1134	*Rhizopogon evadens*	*Pinus ponderosa*
6	EM-1131	*Melanogaster varigatus*	*Arctostaphylos viscidia*
7	EM-1110	*Rhizopogon vinicolor*	—
8	EM-1135	*Rhizopogon elleane*	*Pinus ponderosa*
9	EM-1241	*Suillus grannulatus*	—
10	EM-1243	*Suillus luteus*	—

New approaches

Intraspecific genetic variation in ectomycorrhizal fungi

Isozyme variation and somatic incompatibility were investigated by Karkouri K E L, Layet Marel J C, and Mousain D (1996) (*The New Phytologist* **134**(1): 143–153) to study intraspecific variation and to identify genets in natural populations of the ectomycorrhizal fungus, *Suillus collinitus*. Forty-three isolates obtained from basidiocarps collected from 11 forest sites under *Pinus* trees were examined. Isozyme analysis indicated high intraspecific variation between isolates from the same location and from different locations. Phenetic analysis based on calculated dissimilarity values derived from isozyme landing patterns separated the isolates into two main groups (I and II) with 35% dissimilarity. A specific and highly active acid phosphatase isoform (ACP–2) was detected in most of the group II isolates. Dikaryotic isolates displaying isozyme similarity coefficient of 100% were found to be somatically compatible and were considered to originate from the same genet. The same genet was never found at more than two forest sites. The study showed a high correlation between the results of electrophoretic isozyme analysis and those of somatic analysis combined with somatic incompatibility testing. These are efficient tools with which to study the population biology of ectomycorrhizal fungi found under forest trees.

Oxalate extraction in mycorrhizal root environments

Oxalic acid is secreted by many ectomycorrhizal fungi. It plays a fundamental role in calcareous soils by trapping calcium and releasing phosphorus. Piombo et al. (1996) propose a simple and quick method for calcium oxalate solubilization by mixing 5 g of soil for one hour with boiling one molar hydrochloric acid, the volume depending on sample calcareousness as well as aluminium and iron content, and adding a reducing agent (hydroxylamine) of Fe^{3+} (*Soil Testing and Plant Analysis* **27**(5–8): 1663–1667). Fast staining with H^+ resin put the resulting solution in good condition for ionic chromatographic analysis. The rate of recovery of calcium oxalate added to soils (0.8%) having various calcareous contents (28%–50%) was 93%–101%. The method allowed detection of oxalate as low as 0.01% in 30% calcareous soils with a good reproducibility and was successfully applied to calcareous soils sampled around roots colonized by mycorrhizas.

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