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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI, now The Energy and Resources Institute, 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—the 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 sharing of information 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.

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Role of mycorrhiza in reclamation of mine spoils

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Mining activities involve the removal of the most microbiologically active portion of surface soil and its dumping in open spaces. This operation affects the activities of useful microorganisms including mycorrhizal fungi. Reinoculation of mine spoils with mycorrhizal propagules or with stored surface soil has been found to help in reclamation of mine spoils through their revegetation.

Effect of top soil storage on viability of mycorrhizal propagules in surface mined areas

Studies conducted at the Department of Soil Science and Plant Nutrition, School of Agriculture, The University of Western Australia, Nedlands on four mining sites showed that topsoil disturbance decreased the number of spores or the number of spore types that could be isolated from the soil. The formation of VA mycorrhiza was also reduced or delayed. Subterranean clover and *Acacia* spp. were used as bioassay plants. Greenhouse studies indicated that both, disturbance and period of storage without plant growth contributed to the loss in infectivity of propagules of VA mycorrhizal fungi. After 4–5 years of revegetation at four mining sites, the number of infective propagules appeared to be restored to a level equivalent to that of undisturbed soils (Jasper, Robson, and Abbott 1987).

In studies conducted at the Environmental Research Division, Argonne National Laboratory, Argonne, Illiwis, USA, survival dynamics of VAM fungi were determined, using a bioassay procedure, for soils stored from 0.5–6.0 years in topsoil stock piles associated with a coal surface-mine in the western USA. Propagule mortality could best be related to in situ soil moisture potential using a piece-wise regression model with the breaking point occurring at –2 MPa. The addition of length of storage time contributed significantly to the accuracy of the model. In addition, the piece-wise nature of the data suggested two separate populations of VAM fungi: propagules found in soils with moisture potentials less than –2 MPa and those occurring in soils with moisture potentials greater than –2 MPa. Soil moisture and length of storage time had different effects on each of these populations. When water potential was less than –2 MPa, moisture was an important predictor of inoculum while length of storage had little predictive capability (Miller, Carnes, and Moorman 1985).

In studies conducted at the Department of Range Science and Ecology Center, Utah State University, Logan, Utah, USA, on spoil spore counts of VAM fungi, VAM root infection, and percent cover of plants were assessed on reclaimed soil and spoil (1–6 years old), orphan spoil (10–31 years old), and undisturbed native sites. No relationship was found between site age and spore density. Topsoiled sites almost always had significantly higher spore counts than spoil sites (reclaimed or orphan), indicating the importance of substrate on spore colonization and reproduction. Percent root infection of *Agropyron smithii* and *A. dasystachyum* showed no increasing trends with time in the mined sites (1–31 years), but the plants in the undisturbed site had greater infection than in any of the mined sites. There was also no correlation between percent cover of *Agropyron* and root infection or spore count (Waaland and Allen 1987).

Studies conducted at the Department of Microbiology, North Dakota State University, Fargo, North Dakota, USA, showed that stored topsoil from strip-mining operations contained little or no VAM fungal inoculum at a depth of 120

* Compiled from TERI database – RIZA

cm. Inoculum soil from a native prairie was mixed into the stored soil at the rates of 10 per cent and 1 per cent and surface soil applied at 1 per cent using sterilized inocula as controls. After 30 days growth, the percent VAM fungal infection of maize, the test plants, increased with both 10 per cent and 1 per cent soil inocula. Phosphorus concentrations were generally increased by inoculation with 10% soil mixtures but not with 1 per cent. Shoot dry weights were not measurably different between 10 and 1 per cent inoculation. However, when plant growth period was increased to 60 days, all three parameters increased over the check plants and there was no difference between any of the parameters when the inoculum was mixed or surface-layered (Saxerud and Funke 1991).

Reclamation of surface mined spoils

Reclamation with VAM fungi

Studies were conducted at the Department of Botany, University of Wyoming, Laramie, Wyoming, USA, on *Artemisia tridentata* (big sage brush), inoculated or not with VA-mycorrhizal fungi (present at the site before surface coal mining) and planted on the field site both as seed and container-grown seedlings. Native VA-mycorrhizal endophytes produced only limited mycorrhizal infection in the disturbed soil in the site after mining (approximately 20% of the total root length). Also, they were not found to have a significant effect on the establishment, growth, and survival of sagebrush plants (Stahl, William, and Christensen 1986).

Studies were conducted at the School of Science Technology, Riverina-Murray Institute of Higher Education, Wagga Wagga, New South Wales, Australia, on onion (*Allium cepa*) plants, infected by *Glomus mosseae* in unsterilized coal spoil containing indigenous VAM fungi (including *G. mosseae*). The number of onion roots converted to mycorrhizas by inoculant fungus, estimated by the gridline intersect method, increased with increasing inoculum density ($r = 0.62$, $P = 0.05$) until a plateau was reached. Onion growth response also increased significantly ($P= 0.05$) with the amount of VAM inoculum present in the coal spoil. The initial linear relationship between inoculum propagules (MPN estimates), percent colonization of onion roots, and onion shoot dry weight became quadratic as the number of infection propagules increased. VAM infection had no significant effect on root-shoot ratios. Similarly there was no significant interaction ($P=0.05$) between the inoculum density, VAM-colonized root mass and the onion root-shoot fresh weight ratios. The amount of the windswept bituminous coal spoil bound to VAM (presumably because of VAM external hyphae) also increased as inoculum density increased ($r = 0.63$, $P = 0.05$). There were negative correlations ($P = 0.05$) between root fresh weight per plant ($r = – 0.68$), inoculum density ($r = –0.589$), percent root length infected ($r = – 0.73$) and the amount of spoil adhered per gram root fresh weight (Khan 1988).

Studies conducted at the Department of Plant Sciences, University of Wyoming, Laramie, Wyoming, USA, on development of VAM and proliferation of roots of western wheat-grass (*Agropyron smithii*) in a revegetated mine soil showed that total rooting density (root length per volume of soil) and spore frequency increased as a function of age of reclamation (time elapsed since reseeding). Topsoil replaced during reclamation was apparently the primary source of mycorrhizal inoculum in the most recently reclaimed site. Seedlings growing on spoil material without topsoil were generally non-mycorrhizal, but mycorrhizal rooting density was not significantly correlated to the residual content of soil organic matter (an indicator of the proportion of topsoil in the growth medium). Mycorrhizal colonization in sites up to three years old were minimal in spite of rapid increases in overall rooting density. Mean rooting density, mean degree of infection, and mean spore frequency in older sites (5–7 years) were comparable to those of an adjacent undisturbed rangeland, but were highly variable. Mature, non-mycorrhizal specimens were observed, suggesting that *A. smithii* is not highly dependent on mycorrhizal infection for survival on this disturbed site (Loree and Williams 1987).

Studies conducted at the Botany Institute, Czechoslovakia Academy of Sciences, Pruhonica, Czechoslovakia, showed that in *Acer pseudoplatanus* stands growing on two mine spoils, the quantity as well as species diversity and viability of VAM fungal populations were disturbed. Artificial mycorrhization could improve the growth of seedlings of deciduous trees intended for afforestation of disturbed localities (Vosatka 1990).

Studies conducted at the Department of Botany, Charles University, Benatska, Prague, Czechoslovakia, on plants, dominants of primary succession, on strip coal mining sites, confirmed a correlation between dependence of these plants on mycotrophic nutrition and plant life strategy (Kostkova and Cudlin 1990).

In studies conducted at the Department of Forestry, Wildlife and Fisheries, Agricultural Experiment Station, University of Tennessee, Tennessee, USA, 19-week old seedlings of yellow poplar (*Liriodendron tulipifera*), inoculated or not with *Glomus mosseae* or *G. fasciculatum* , were planted on three sites: a previously wooded site, an abandoned field or stripmine spoil. Survival of seedlings after 1, 2, and 4 years and heights and root collar diameter were significantly greater in VAM treated seedlings than the controls (65% survivals in VAM versus 46% in non-VAM treatments after 4 years), with minimal competing vegetation. *G. fasciculatum* colonized seedlings were consistently larger in all treatments (Hay, Rennie, and Ford 1989).

In studies conducted at the Central Arid Zone Research Institute, Jodhpur, Rajasthan, India, out of 31 plant species occurring naturally on surface mine spoils, 10 species were selected for their establishment on surface mine spoils after inoculation with *Glomus fasciculatum* (isolated from mine spoils) at the rate of 10 spores per gram of soil. Percent infection increased 2-9 fold in inoculated plants. Shoot biomass, N, P, K, Zn, and Cu concentrations were significantly improved over normal in all cases (Rao, Tarafdar, and Tak 1999).

Studies conducted at the All-Russian Agricultural Biotechnology Research Institute, Moscow, Russia, on growth of alfalfa on ameliorated sulphide containing soils from a brown coal mine area showed that the treatment of alfalfa seeds by fusicoccin prior to planting led to the two times enhancement of the development of intraroot structures (arbuscules) of endomycorrhizal fungi. Joint employment of fusicoccin and stress adapted fungus, *Glomus mosseae*, increased the alfalfa green biomass by 1.8 times comparing to controls (Zolnikova, Serebrennikova, Leonova, and Muromtsev 1997).

Reclamation with ectomycorrhiza

Studies conducted at the Institute for Mycological Research on Development, United States Department of Agriculture, Forest Service, Southeastern Forest Experiment Station, Athens, Georgia, USA, showed that revegetation of disturbed lands such as acid coal spoils, kaolin spoils, burrow pits, and severely eroded sites was significantly improved by planting tree seedlings tailored in the nursery with *Pisolithus tinctorius*. *P. tinctorius* mycorrhizae on disturbed sites promoted the assimilation and absorption of required nutrients, increased water absorption, reduced the absorption of toxic elements and, apparently reduced the overall stress inherent at these sites (Marx and Cordell 1975).

Studies conducted at the Division of Reclamation, Ohio Department of Natural Resources, Columbus, Ohio, USA, showed that traditional practices for reclaiming abandoned mined land are very expensive ($7000/per acre) but reclamation through reforestation costs only $300/– per acre. The success of the reforestation programme on sites of area 2–75 acres is attributed to the use of tree seedlings inoculated with the ectomycorrhizal fungus, *Pisolithus tinctorius*. Inoculated Virginia pines (*Pinus virginiana*) planted in 1986, on barren shales (pH 2.8) had 99% survival, mean DBH 2.9 cm, and mean height 12.9 cm in 1989 (Caldwell 1990).

Studies were conducted at the Department of Forestry, Wildlife and Fisheries, Agricultural Experiment Station, University of Tennessee, Tennessee , USA, to determine the effect of mycorrhizal (*Pisolithus tinctorius*) inoculation and discing (to reduce competition) on survival and height of container grown seedlings of Loblolly pine (*Pinus taeda*) and Virginia pine (*Pinus virginiana*), planted on various dates between May 21 and September 24, 1982 on a reclaimed strip mined site. Survival and growth after 5 years were best with combined mycorrhizal inoculation and discing and for tree seedlings planted earlier in the season (Mullins, Buckner, Evans et al. 1982).

Reclamation with mycorrhiza and phosphorus fertilization

Field trials conducted at the Native Plants Inc., Salt Lake City, USA, on inoculation of Salix viminalis and *S. dasyclados* with ectomycorrhiza for biomass production on surface-mined peatlands in northern Finland revealed (after one growing season) statistically significant growth stimulation due to mycorrhiza formation in both species. Plants inoculated with *Entoloma* were sometimes twice as large as the control plants. However, such effects were observed only in plants receiving normal phosphate fertilization as opposed to low phosphate application, and were not consistent from season to season. With the inoculum of other species (*Cortinarius purpurascens, Hebeloma crustuliniforme, and Paxillus involutus*), some evidence of growth enhancement was found in the field, but these results were sometimes attributable to the non-symbiotic effects of inoculation. In greenhouse experiments, both species formed ectomycorrhizas with *Amanita* spp., *Cortinarius purpurascens, Entoloma nidorosum, Entoloma spp., Hebeloma crustuliniforme, Hebeloma pusillum, Laccaria bicolor, Paxillus involutus* (Loree, Lumme, Neimi et al. 1989).

In studies conducted at the Mulga Research Centre, School of Environmental Biology, Curtin University of Technology, Bentley, Perth, Australia, seedlings of *Allocasuarina fraseriana* and *Actinostrobus pyramidalis* were grown in coalmine dumps, inoculated with *Pisolithus tinctorius* or *Glomus fasciculatum* and supplied with different levels of phosphorus (0, 10, 20, 40, and 80 kg per ha). *A. fraseriana* was highly sensitive to all levels of phosphorus and mortality was at least 60% at all phosphorus treatments after 8–9 weeks. Plants did not respond to mycorrhizal inoculation. *A. pyramidalis* formed mycorrhiza with *G. fasciculatum*, but infectivity was very low and was greatest at 20 kg phosphorus per ha and at this treatment, the yield was best. *A. fraseriana* formed ectomycorrhizae in both dumped and non-dumped situations. Mycorrhizal infectivity was greater in seedlings of natural habitat than in seedlings grown on an abandoned mine waste dump but nutrient concentrations did not differ (Alvin, Fox, and Schatral 1992).

Reclamation with mycorrhiza and nitrogen fertilization

Studies conducted at the Environmental Resources Management, Inc. Exton, USA, on big toothed aspen (*Populus grandidentata*) seedlings, inoculated or

not with *Pisolithus tinctorius* and transplanted to an acidic, infertile abandoned mine soil showed 71 per cent survival after one year. Nitrogen-fertilized, inoculated seedlings had the lowest survival rate. Growth of aspen after 15 months showed no difference between uninoculated and inoculation treatments. The only significant growth increase occurred with treatments of nitrogen and phosphorus (DeMuro, Jencks, and Hindal 1990)

Reclamation with mycorrhiza and N P K fertilization

With ectomycorrhiza

In studies conducted at the Department of Range, Wildlife and Forestry, University of Nevada, Reno, Nevada, USA, one-year old bare-rooted seedlings of loblolly pine (*Pinus taeda*), inoculated or not with *Pisolithus tinctorius* were out planted on a surface-mined site, previously contoured and hydro seeded with a mixture of herbaceous ground cover species. Control seedlings were naturally infected by *Thelephora terrestris*. N P K fertilizers (336 kg per ha) were broadcast at outplanting on half the plots. After 7 years, survival and growth of trees previously inoculated with *P. tinctorius* were significantly improved in relation to the control plants. Fertilizer application elicited a significant reduction in survival and a negligible growth response on trees of both mycorrhizal treatments, due primarily to its stimulation of competing herbaceous species. During the third growing season, xylem pressure potential of seedlings with *P. tinctorius* was significantly less negative than that of control seedlings during a prolonged period of moisture stress. Analyses of foliar samples collected during the third growing season revealed that the seedlings infected by *P. tinctorius* had more NO_3 and less Zn in their needles than control seedlings (Walker, West, McLaughlin et al. 1989).

With VAM

Studies conducted at the N Pushkarov Institute of Soil Science and Yield Programming, Sofia, Bulgaria, showed that germination and yield of *Dactylis glomerata* on coal-mined land were improved by upto 150% by inoculation with mycorrhizal and nitrogen-fixing microorganisms in the presence of 200+200+200 kg N+P+K. The organisms used were *Glomus mosseae*, *G. gerdemannii*, *Gigaspora margarita*, and *Azospirillum* (Taleva and Dimitrova 1990).

In studies conducted at the Department of Environmental Biology, University of Guelph, Ontario, Canada, yellow poplar (*Liriodendron tulipifera*) seedlings were raised in nursery beds with four mycorrhizal treatments (no inoculum, *Glomus* spp., *G. mosseae*, natural inoculum) and four fertilizer treatments (no fertilizer, 15-15-15, 560 kg/ha as base fertilizer, base+two urea top dressings at the rate of 39-0-0, 112 kg/ha, base+4 urea top dressing) for one year. The seedlings were outplanted at three different sites (a reclaimed strip-mined site, an eroded old-field site with shallow to shale bedrock, a river terrace site). Fertilizer treatments significantly affected the size of the seedlings in the nursery but not their post planting growth. Mycorrhizal inoculation did not greatly affect seedling size in the nursery but it affected post planting height growth. The seedlings inoculated with *Glomus* spp. grew the most followed by seedlings inoculated with natural inoculum, with *G. mosseae* and no inoculum treatments. Seedling survival and foliar concentration of nitrogen and phosphorus were not significantly impacted by treatments in the nursery. Seedling growth was best at the river terrace site and worst at the eroded field site. Differences in seedling growth at the river terrace site due to soil quality overshadowed differences due to nursery mycorrhizae and fertilizer treatments. Glomus inoculated seedlings grew better under harsher conditions but those inoculated with soil from a natural stand did almost as well (Williams and Lietzke 1993).

Reclamation with mycorrhiza and organic amendments

In studies conducted at the Institute for Mycorrhizal Research, United States Department of Agriculture, Forest Service, Southern Forest Experiment Station, Forestry Sciences Laboratory, Athens, Georgia, USA, a burrow pit was graded and subsoiled. Out of 30 plots, thus prepared approximately $1m^3$ dried sewage sludge was spread evenly over15 plots. On the remaining 15 plots, 10-10-10 (NPK) fertilizer (3 kg per plot) and dolomite (12 kg per plot) were broadcast. Four month old, loblolly pine seedling raised on peat-vermiculite growing medium, inoculated either with *Pisolithus tinctorius* or *Thelephora terrestris* or uninoculated were planted on these plots (25 seedlings in each plot). Among plots receiving sewage sludge, *P. tinctorius* colonized seedlings had significantly greater height, stem collar diameter and seedling volume than *T. terrestris* or control seedlings after two years growth. Among plots receiving fertilizers, the above parameters were not different between *P. tinctorius* or *T. terrestris* seedlings but in *P. tinctorius* seedlings, these parameters were greater than in the control. After planting, seedlings colonized by *P. tinctorius* survived better than *T. terrestris* or controls on fertilized plots but there was no difference with sewage sludge treated plots (Ruehle 1979).

In studies conducted at the Department of Biology, the University of Calgary, Calgary, Alberta, Canada, *Agropyron trachycaulum* (slender wheat) was planted in wooden tanks filled with mine spoil, either from a coal strip-mine located in a subalpine spruce fir habitat or extracted oil sands and amended either with fertilizer or fibrous peat,

or sewage sludge or kept unamended. In peat amended soil, endomycorrhiza was detected after two weeks, in control and fertilized treatments by six weeks, and in sewage amended soil by ten weeks. At 10 weeks after emergence, there was no significant difference in the length of infected roots per plant among the amendments (Zak and Danielson 1979).

In further studies conducted at the above University, container grown jackpine seedlings were planted in extracted oil sands amended with either fertilizer or fibrous peat or sewage sludge or kept unamended. Roots after planting were heavily infected with *Thelephora terrestris*. After first growing season, roots grown in soil were mycorrhizal only in peat amendment. After two growing seasons 24% to 72% of roots were mycorrhizal. Plants grown in the peat amended had significantly higher root infection than other amendments. *T. terrestris* was the dominant fungus in all treatments except the one with peat amendment where ectendotype mycorrhiza dominant. Suillus was found in all amendments except sewage. After two growing seasons, plants in sewage amended soil were 13 times heavier than the controls and 4 times heavier than those grown with peat and fertilizer amendments (Danielson and Zak 1979).

In studies conducted at the Washington State University, Department of Natural Resource Science, Pullman, Washington, USA, blue bunch wheat grass (*Pseudoroegneria spicata* x *Elymus lanceolatus* ssp. *Lanceolatus*) was grown in non-sterile mine spoil amended with 0.0, 6.2, 12.4, 24.5, and 49.0 g dry composted sewage sludge (CSS) per litre of spoil-sand mix for 16 weeks in a glasshouse experiment. The VAM treatments were spores of *Glomus mosseae* collected from native blue bunch wheatgrass plants, non-native spores of *Glomus intraradices* and *Entrophospora* or no added spores. The plants also grown in non-sterile, stockpiled topsoil with both AMF treatments to compare to AMF present in the topsoil. Each CSS rate increase produced increased above ground and below ground plant growth. The plant growth was enhanced by native AMF in CSS amendment spoil but CSS appeared to reduce AMF colonization; however, hyphae levels in plants with native AMF were unaffected. Plant growth was not affected by AMF treatments in stockpiled topsoil but AMF colonization was greater in plants with the topsoil alone (Thorne, Zamora, and Kennedy 1998).

Interaction of mycorrhiza and acid producing bacteria in mine spoils reclamation

Studies conducted at the Henderson Community College, University of Kentucky, Henderson, Kentucky, USA, showed that *Pisolithus tinctorius* introduced to acidic mine spoil on infected pitch pine (*Pinus rigida*) seedlings had no effect on population or activity of *Thiobacillus* spp., although there may have been a rizosphere effect. Apparently, spoil acidification by bacterial catalysis of pyrite oxidation cannot be controlled by transplanting *P. tinctorius* infected tree seedlings on mine spoil, and the inhibition of these bacteria is not the mechanism by which *P. tinctorius* adapts its host to acidic sites. Evidence was obtained that a complete N P K fertilizer stimulated the activity of *Thiobacillus* spp. in a sulphur amended mine spoil. Soluble and slow release fertilizers in conjunction with sulphur produced similar effects on acid production and related events. Acid generation in sulphur amended spoil appears to involve *T. thioxidans* but not *T. ferrooxidans* (Hunt, Hendrix, and Maronek 1986).

Studies conducted at the Department of Plant Pathology and Horticulture and Landscape Horticulture, University of Kentucky, Lexington, USA on loblolly pine seedlings transplanted on pyritic coalmine site for a commercial reclamation effort showed that after five years, trees which became naturally infected with *Pisolithus tinctorius* or other ectomycorrhizal fungi which produced sporocarps, were twice the height and stem diameter of trees not associated with sporocarps. Soil pH was lower and sulphate was higher in the root zones of trees associated with *P. tinctorius* than with trees not associated with *P. tinctorius*. Differences were not found in ferric or ferrous ions or in populations of the two bacterial species, *Thiobacillus ferroxidens* and *T. thioxidens* which ctalyse production of sulphuric acid from pyrite. Apparently *P. tinctorius* benefits its hosts by mechanisms other than inhibition of *Thiobacillus* spp. (Hendrix, Hunt, and Maronek 1986).

Reclamation with rooted cuttings and mycorrhiza

In studies conducted at the University of Alaska, Fairbanks, Palmer, Alaska, rooted cuttings of *Populus balsamifera* and seedlings of *Alnus crispa*, grown in sterile soil, were outplanted on abandoned mine spoils inoculated with soils from early aged, intermediate aged, and mature forests. Both early aged and intermediate aged forests contained *P. balsamifera* and *A. crispa* while mature forests had many additional plant species. Plant height and basal diameter were measured at the beginning and end of each growing season and after two years growth. Individuals of *A. crispa* inoculated with soil from mature forest produced twice as much current growth during year 2 and contained higher nutrient concentrations in leaf tissue when compared with individuals inoculated with soil from young stands. Individuals of *P. balsamifera* treated with the soil from the mature community were slightly larger than those treated with the soil from the young community. *P. balsamifera* is a pioneer in primary succession while *A. crispa* occurs slightly later in primary succession and becomes established on secondary

disturbance. Mycorrhize which infected *A. crispa* were predominantly a brown, woody type while other types accounted for greater relative mycorrhizal infection percentage on *P. balsamifera* (Helm, and Carling 1990, 1993b)

Further studies conducted at the above University on rooted cuttings and volunteer plants of *Populus balsamifera* raised on an abandoned mined site which was inoculated with soil from a native plant community showed that plant height was significantly increased only when soil transfer and additional P treatments were combined. Response to addition of P alone or soil transfer alone were not significantly different from each other. Current twig growth increased with either treatment alone or both combined. Soil transfer on cuttings resulted in more ectomycorrhizal formation than either the control or additional P. Leaf nitrogen concentrations in cuttings and volunteers increased when plants were treated with soil transfer. Phosphorus added to the base level of N P fertilizer plus soil transfer did not suppress or improve mycorrhizal formation compared to that with soil transfer alone (Helm and Carling 1993a).

Revegetation of uranium mine spoils

Studies conducted at the CSIRO, Division of Forestry, Western Australia, on revegetation of waste rock dumps at a uranium mine showed that ecotomycorrhizae are a key component of the restoration of degraded sites with tree species (Malajczuk, Reddell, and Brundrett 1994).

Revegetation of phosphate mine spoils

In studies conducted at the Soil Science Department, University of Florida, Gainsville, Florida, USA, rooted cuttings of *Cornis foeminia*, *Ilex glabra*, *Clethra alnifolia*, grown in pasteurized or non-pasteurized phosphate mine spoil in a shade house were inoculated with *Glomus intraradices*, *G. etunicatum* or a mixed *Glomus* culture that originated from a 10-year old mine reclamation site and were harvested after eight months. In pasteurized soil, VAM fungal inoculation increased shoot and root masses of *C. foeminia* and *I. glabra* but not those of *C. alnifolia*. The intensity of VAM fungal inoculum appeared to be controlled by the plants species. In non-pasteurized soil, plant size was generally less than in pasteurized soil, and there was no growth response to VAM fungal inoculation (Sylvia 1988).

In further studies conducted at the above University, cuttings of *Aronia arbutifolia*, *Clethra alnifolia*, *Cornus foemina*, *Ilex glabra*, and *Viburnum nudum* inoculated in a rooting bed with *Glomus etunicatum*, *Glomus intraradices* or a mixed culture containing several unidentified *Glomus* spp. recorded a low level of root colonization (overall mean of 2 per cent excepting *V. nudum*) after seven weeks. These low percentages were sufficient to obtain well colonized plants (overall means 57%) after rooted cuttings were transplanted to potting medium and grown in a shade house for 7 months. Inoculation had no effect on plant growth or survival after outplanting to a site that contained abundant clay aggregates and where controls became colonized by VAM (Sylvia 1990).

Reclamation of iron ore spoils

Studies conducted at the Soil Science and Plant Nutrition, School of Agriculture, University of Western Australia, on revegetation of iron ore mining soil showed that VA mycorrhizae were formed only in soils from sites dominated by *Triodia pungens, Acacia pyrifolia* nodulated only in soil from sites dominated by *A. aneura*. In low-grade ore, phosphorus deficiency was the major limitation to plant growth. Inoculation with *Glomus* sp. resulted in up to 70% increases in dry matter production at low rates of phosphorus application. Also, response to phosphorus or inoculation with VA mycorrhizal fungi was limited by nitrogen deficiency (Jasper, Robson, and Abbott 1988).

In glass house experiments conducted at the Department of Plant Pathology, University of Minnesota, St. Paul, Minnesota, USA, using coarse taconite iron ore tailing as the substrate, interactions between the warm season grasses, *Andropogon gerardii* (big bluestem) and *Schizachyrium scoparium* (little bluestem) and the cold season grass, *Elymus canadensis* (Canada wild rye) and indigenous VAM fungi from an adjacent fine tailing basin were examined. *A. gerardii* was highly dependent and responsive to inoculation at low P, whereas inoculation had no significant effect on the growth of *S. scoparium*. Root dry matter and root lengths of both warm-season grasses were unaffected by mycorrhizal inoculation. *E. canadensis* was responsive only at the lowest P level. Shoot dry matter, P uptake, root dry matter, and root length were all greater in the control than in inoculated plants at moderate P levels. Despite a growth suppression, colonized root lengths for *E. canadensis* were approximately five times more than warm season grasses at low P levels (Noyd, Pfleger, and Russelle 1995).

Further studies conducted at the above University on reclamation of taconite iron ore tailing seeded with a mixture of native prairie grasses, showed that plant cover and biomass, percent seeded species, mycorrhizal infectivity, and spore density were greatly increased by additions of composted yard waste. After three seasons, total plant cover was also greater in plots with added fertilizer. Third season plant cover was greater in plots amended with the higher rate of compost (44.8 Mg/ha) than the moderate rate (22.4 Mg per ha). Field inoculation with AM fungi also increased plant cover during the second season and infectivity during the first two seasons. Dispersal of AM fungal propagules into

non-mycorrhizal plants occurred rapidly and increased infectivity in compost amended plots during the third season. In plots with less than 10% plant cover, AM fungal infectivity of inoculated plots was greatly reduced after the second season. (Noyd, Pfleger, and Norland 1996).

In studies conducted at the N O Arizona University, Centre for Environmental Science and Education, Flagstaff, Arizona, USA, *Panicum virgatum* (a late successional grass planted during reclamation) and *Salsola kali* (an early successional colonizer of taconite tailings) were grown in taconite tailings along an experimental phosphorus gradient with or without mycorrhizal inoculum isolated from reclaimed taconite tailings. At low phosphorus concentrations, myocrrhizal inoculum enhanc ed the height and drymass of *Panicum* but the growth decreased at the two highest phosphorus concentrations. Mycorrhizal inoculm did not enhance the growth of *Salsola* at any phosphorus level but the growth was decreased at the highest phosphorus concentrations. In field plots, mycorrhizal inoculum and organic soil amendment (composted paper mill sludge) enhanced the growth of *Panicum* and decreased the growth of *Salsola* (Johnson 1998).

Reclamation of lignite over burdens

In studies conducted at the Department of Range Science, Texas A and M University, College Station, Texas, USA, seedlings of side oats (*Boutelona curtiendula*), Indian grass (*Sorghastrum nutans*), and Kleui grass (*Panicum coloratum*) were inoculated with VAM fungi (*Glomus fasciculatum* and *Gigaspora margarita*) in a containerized system and transplanted into lignite overburden. After three growing seasons, without cultural inputs, plants inoculated with VAM fungi had greater survival percentages, basal diameters, and above-ground biomass, had higher level of nitrogen and phosphorus in above-ground biomass and had fairly stable root colonization percentages than non-inoculated plants which showed low levels of colonization over the three-year study period (Call and Davies 1988).

In studies conducted at the Department of Horticulture of the above university, seedlings of live oak (*Quercus virgininiana*), Chines tallow tree (*Sepium sebiferum*), and Texas mountain laurel (*Sophora secundiflora*) were inoculated with either ectomycorrhizal fungus (*Pisolithus tinctorius*) or VAM fungi, *Glomus fasciculatum*, *Gigaspora margarita*, and *Glomus mosseae* in a containerized system and transplanted into lignite overburden at two separate mine sites. Ectomycorrhizal *Q. virginiana* and VAM infected *S. sebiferum* exhibited greater growth and VAM inoculated *S. secundiflora* showed greater survival and growth than non-inoculated controls. Root colonization of inoculated plants was high at both sites with low P as well as with moderately high P while non-inoculated plants had low levels of colonization. Thus both ectomycorrhizal and VAM fungi enhanced growth of the three woody species in these nitrogen-deficient overburden sites, independent of overburden P (Davies and Call 1990).

Studies conducted at the Departmento de Fisiologia Vegetal, Instituto de Investigaciones Agrobiologicas de Galicia, Santiago de Compostela, Spain, on *Festuca pratensis* and *Trifolium pratense* grown on a composite lignite mine spoil under greenhouse conditions and inoculated with five VAM fungal inocula showed toxic levels of Al, Mn, and Zn in plant tissues, with *F. pratensis* showing apical necrosis. These mine spoils had a high potential acidity and poor physical and chemical conditions for plant growth. *Glomus deserticola* was the only VAM fungus that succeeded in colonizing plant roots, although it failed to improve the etablishment or development of either species (Arines and Vilarino 1991).

Reclamation of tin mine over burdens

Studies conducted at the Forest Pathology and Microbiology, Royal Forest Department, Bangkok, Thailand on reforestation of tin-mined soils showed that the growth of *Eucalyptus camaldulensis* measured by increase in plant height, diameter and root collar, and total biomass, was greatest by manure fertilization followed by ectomycorrhizal inoculation with *P. tinctorius* plus manure. Growth of *Pinus caribaea* var *hondurensis* was increased most by ectomycorrhizal inoculation plus manure, followed by ectomycorrhizal inoculation alone. Too much manure increased soil pH and reduced the growth of *P. tinctorius*. Thus ectomycorrhizal inoculation and manure fertilization promote vigour and survival of seedlings before transplanting to reforest old tin mine areas (Aniwat and Somboon 1990).

Revegetation of kaolin spoils

Studies conducted at the Department of Biology, Virginia Polytech Institute and State University, Blacksburg, Virginia, USA, on reforestation of kaolin spoils with sweetgum (*Liquidambar styraciflua*) inoculated or not with *Gigaspora margarita* showed that after 161 days, non-mycorrhizal seedlings grew best in a soil supplemented with 100 ug P per g of soil but in kaolin spoil, these plants did not respond to this rate of fertilization unless they were mycorrhizal. In soil receiving 25 ug P per g, growth rates of mycorrhizal seedlings were significantly greater than that of non-mycorrhizal seedlings. Foliar levels of phosphorus, magnesium, and calcium were enhanced by both mycorrhizal colonization and P fertilization. Mycorrhizal development and spore production were suppressed at the high P level (Gruhn, Roncadori, and Kormanik 1987).

Reclamation of bauxite mine spoils

Studies conducted at the Soil Science and Plant Nutrition, School of Agriculture, the University of Western Australia, Nedlands, Australia, showed that during bauxite mining in *Eucalyptus marginata* forest, the infectivity of propagules of VAM fungi in topsoil was destroyed, even when the soil was stripped and spread promptly without stockpiling. Most infectivity was lost within three weeks of clearing the vegetation, before the soil was disturbed. The rapid loss of infectivity may be associated with the absence of spores in the soil. In a revegetated, respread soil, the infectivity of VA mycorrhizal fungi was substantially greater than that of freshly disturbed soil, but lower than levels recorded in similar undisturbed forest soil. There were no clear seasonal changes in VA mycorrhizal infectivity, indicating that the loss of infectivity during mining was not a seasonal response. In a greenhouse experiment, dry matter production by *Acacia pulchella* in soil disturbed during bauxite mining was at least doubled if the soil was inoculated with effective VA mycorrhizal fungi (Jasper, Abbott, and Robson 1989).

Reclamation of chromite mine spoils

In studies conducted at the Laboratory of Microbiology and Plant Pathology, Post Graduate Department of Botany, Utkal University, Bhubaneswar, Orissa, India, cowpea (*Vigna unguiculata*) plants were grown in polypots containing chromite overburdened soil and inoculated with four VAM fungi, *Glomus mosseae*, *G. fasciculatum*, *Gigaspora margarita*, and *G. gilmora* cultured in Empicheri grass and applied to soil with root chops. Observations were made at the preflowering stage of plant growth (20 days after sowing). Results indicated that all VAM inoculation increased plant dry weight, *G. mosseae* being most effective, though the infective capacity was highest in *G. margarita*. There was also increase in shoot length with a concomittant decrease in root length except *G. gilmora*, which showed the reverse trend. The numbers, size, and moisture content of nodules increased with VAM infection, but there was reduction in nodular dry weight indicating a negative impact of mycorrhizae in mine soil. The rhizospheric microbial population (rhizobial and other bacterial) were seen to be stimulated in general while the fungal population was depressed. Inoculation with VAM species induced differential responses in cowpea grown in chromite mine waste soil among which *G. mosseae* appeared to be most promising (Mishra, Patnaik, and Padhi 1991).

In further studies at the above University, a comparative assessment on the effect of four VAM species *Glomus mosseae*, *Glomus fasciculatum*, *Gigaspora gilmora* and *Gigaspora margarita* on growth, nodulation and total N content of *Acacia nilotica* was done on chromium mine over burdened soil. A general increase in shoot height, plant dry weight, and total N content of the plant was observed due to all types of VAM inoculation. *G. mosseae* showed highest stimulatory effect in terms of shoot height, plant dry weight, and nodule number while *Gigaspora margaita* treatment showed the least impact on improving biomass and *G. gilmora* on nodulation of the test plant (Thatoi, Padhi, and Misra 1999).

Reclamation of molybdenum mine spoils

In studies conducted at the Department of Forest and Wood Sciences, Colorado State University, Fort Collins, Colorado, USA, seedlings of *Pinus contorta*, *Pinus flexilis*, and *Picea engelmannii*, colonized with *Pisolithus tinctorius*, *Suillus granulatus,* and *Cenococcum graniforme* or uninoculated (colonized by a wild fungus) in nursery were planted at a high altitude (3200 m) molybdenum tailing waste site, which was covered with deep mine waste material. The seedlings were given 4 fertilizer treatments namely no fertilizer, 90 kg/ha P, 90 kg/ha P and 68 kg/ha N, and sewage sludge and woodchips at 45000 kg/ha. After two growing seasons, combined mortality was 26.1%. There was significantly greater mortality in sewage sludge and wood chips treatments in both pines. The effect of mycorrhizal treatment on mortality was not significant. Height growth in all three species was greatest with *S. granulatus* and wild fungus, while height growth of seedlings infected with *P. tinctorius* was generally about one half the growth of the best fungal treatment (Steven, Grossnickle, Reid 1979).

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Research findings

Mass multiplication of *Glomus fasciculatum* using different hosts

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Introduction

Mycorrhizal fungi are key components of soil microbiota. They are obligate symbionts and are not host specific (Bonfante-Fasolo 1987). VAM (Vesicular-arbuscular mycorrhizal) fungi form obligate symbiotic associations with the roots and other underground parts of most plants. Their association with plant roots indirectly resist penetration by nematodes, thus helping the plant in its growth and production. It is well known that VA mycorrhizal fungi improve the growth of plants by providing a layer absorptive surface compared with root hairs, and thus help in the absorption of relatively immobile ions in soil (Bagyaraj 1992). AM (arbuscular mycorrhizae) fungi are ubiquitous in their distribution throughout the plant kingdom (Gerdemann 1968). The present investigation was conducted to develop mass production technologies for AM fungi. This information will be useful to plant breeders and farmers.

Materials and methods

For the present study, different host plants (10) were grown in earthen pots of 30 cm diameter containing sterilized soil. The sandy loam soil had the following properties: pH (6.3), organic carbon (0.38%), total nitrogen (0.06%), available phosphorus 3 ppm, and available potassium (11.2 ppm). Before sowing, the inoculum of AM fungus

(*Glomus fasciculatum*) was added to the planting hole at the rate of 50 spores/kg of soil for multiplication. After one and two months, shoot length, shoot weight, root length, root weight, spores in soil and % root infection were recorded to find a suitable preferred host for the multiplication of *Glomus fasciculatum*. Spores were extracted from soil by the wet sieving and decanting method (Gerdemann and Nicholson 1963), AM infection percentage was also made (Phillips and Hayman 1970).

Results and discussion

Association of AM fungi showed the positive effect of symbiosis in most hosts. *Glomus fasciculatum* inoculated plants were found growing more luxuriantly than the controls and sorghum (*Sorghum bicolor*) was found to be the best host. The AM spore population was 163.2 per 100 grams of soil after two months. This was followed by *bajra* (pearl millet) with a spore population of 160.2 per 100 grams of soil after two months, maize with a spore population of 140.2 per 100 grams of soil. The AM spore population was more than 80 in 100 gram soil of groundnut, gram, pea, and cajan whereas in all the other hosts, the spore count was less than 80. AM percentage infection was higher (58.2) in sorghum compared to other hosts (Table 1).

Shoot length, shoot weight, root length, root weight, spore count, and % infection was higher in graminaceous and leguminous crops compared to other hosts. Sieverding and Leiher (1984) and Sundara Babu, Poornima, and Sunguna (2001) have reported that graminaceous and leguminous crops generally tend to increase AM population whereas other hosts decrease the population of AM fungi.

Table 1 Mass multiplication of *Glomus fasciculatum*: host preference

Host	*Shoot length* (cm)	*Shoot weight* (g)	*Root length* (cm)	*Root weight* (g)	*Spore count/ 100 g soil*	*AM percentage infection*
Sorghum						
Month-I	50.6	17.4	16.3	5.2	20.4	19.0
Month-II	72.3	32.4	18.2	6.3	163.2	58.2
Bajra						
Month I	46.6	14.2	14.2	4.8	21.2	22.0
Month II	68.2	22.4	17.4	5.8	160.2	53.4
Maize						
Month I	44.2	15.0	13.2	5.1	35.4	29.2
Month II	63.4	30.2	14.6	5.4	140.2	52.2
Rice						
Month I	22.8	11.4	11.2	4.8	50.2	23.4
Month II	40.0	18.2	12.8	5.8	94.8	39.6
Gram						
Month I	15.8	8.4	7.4	3.6	40.2	31.4
Month II	20.6	13.2	9.0	3.8	89.4	30.2
Groundnut						
Month I	17.2	6.9	8.8	4.0	91.2	44.2
Month II	20.2	10.4	9.1	4.6	117.4	48.8
Cajan						
Month I	22.6	8.6	8.8	3.8	20.2	40.2
Month II	35.4	13.7	11.4	5.2	80.6	52.4
Pea						
Month I	19.4	9.6	10.1	5.1	48.4	44.2
Month II	26.2	13.8	12.0	5.4	86.2	49.8
Brinjal						
Month I	18.6	8.1	11.1	4.6	60.2	52.2
Month II	30.2	15.4	12.4	5.0	79.1	50.8
Chilli						
Month I	17.4	7.3	12.4	5.1	30.7	42.0
Month II	28.8	18.2	13.2	6.4	68.2	48.4

Mean of three replicates

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Incidence of arbuscular mycorrhizal colonization in tubers of *Gloriosa superba* L.

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Introduction

The majority of the vascular flora has mycorrhizal association with AM (arbuscular mycorrhizae) dominating (Brundrett 1991). The presence of the AM has been reported from different habitats and a wide range of plants (Bhat and Kaveriappa 1997). However, Taber, and Trappe (1982) for the first time reported the presence of AM fungi in underground storage organs. They reported the presence of arbuscular mycorrhizal association in the vascular system of rhizomatous tissue and the scale-like leaves of *Zingiber officinale* L. Later, several papers reporting the incidence of AM fungal colonization in underground storage organs of *Curcuma longa* L. (Sampath and Sullia 1992); *Amorphophallus commutatus* Engler (Rodrigues 1995); *Pueraria tuberosa* (Willd.) DC (Rodrigues 1996), *Colocasia esculenta* (L.) Schott (Bhat and Kaveriappa 1997), and garlic (Kunwar, Reddy, and Manoharachy 1999) have been published.

Gloriosa superba L. is a medicinal plant belonging to the family Amaryllidaceae, used by tribal people and ayurvedic practitioners as an antidote for several ailments. This plant is commonly found in and around the Taliegao Plateau during the wet season. The plant perennates in the form of a tuber during the dry season, and sprouts with the onset of monsoon indicating the vegetative phase. The plant has a short life span of 4–5 months.

The present paper reports the incidence of AM fungal association in tubers of *Gloriosa superba* L. and the diversity of AM fungi associated with it.

Materials and Method

Root samples and the rhizosphere soil samples of *Gloriosa superba* L. were collected during the monsoon in July. The plants were uprooted entirely for the collection of samples.

Freshly collected tubers and roots of *Gloriosa superba* L., were washed in water, cleared with 10% KOH, acidified with 1 N HCl, and stained in 0.05% trypan blue in lactoglycerol (Phillips and Hayman 1970). Quantification of root colonization of AM fungi was carried out using the slide method (Giovannetti and Mosse 1980).

Spores of AM fungi were extracted from the rhizosphere using the wet sieving and decanting method (Gerdemann and Nicholson 1963). Quantification of spore density of AM fungi was carried out using the method given by Gaur and Adholeya (1994).

Diagnostic slides with spores/sporocarps were prepared using polyvinyl alcohol lactoglycerol (PVLF) as mountant. Identification of AM fungi was carried out using relevant literature (Morton and Benny 1990; Schenck and Perez 1990; Wu 1993).

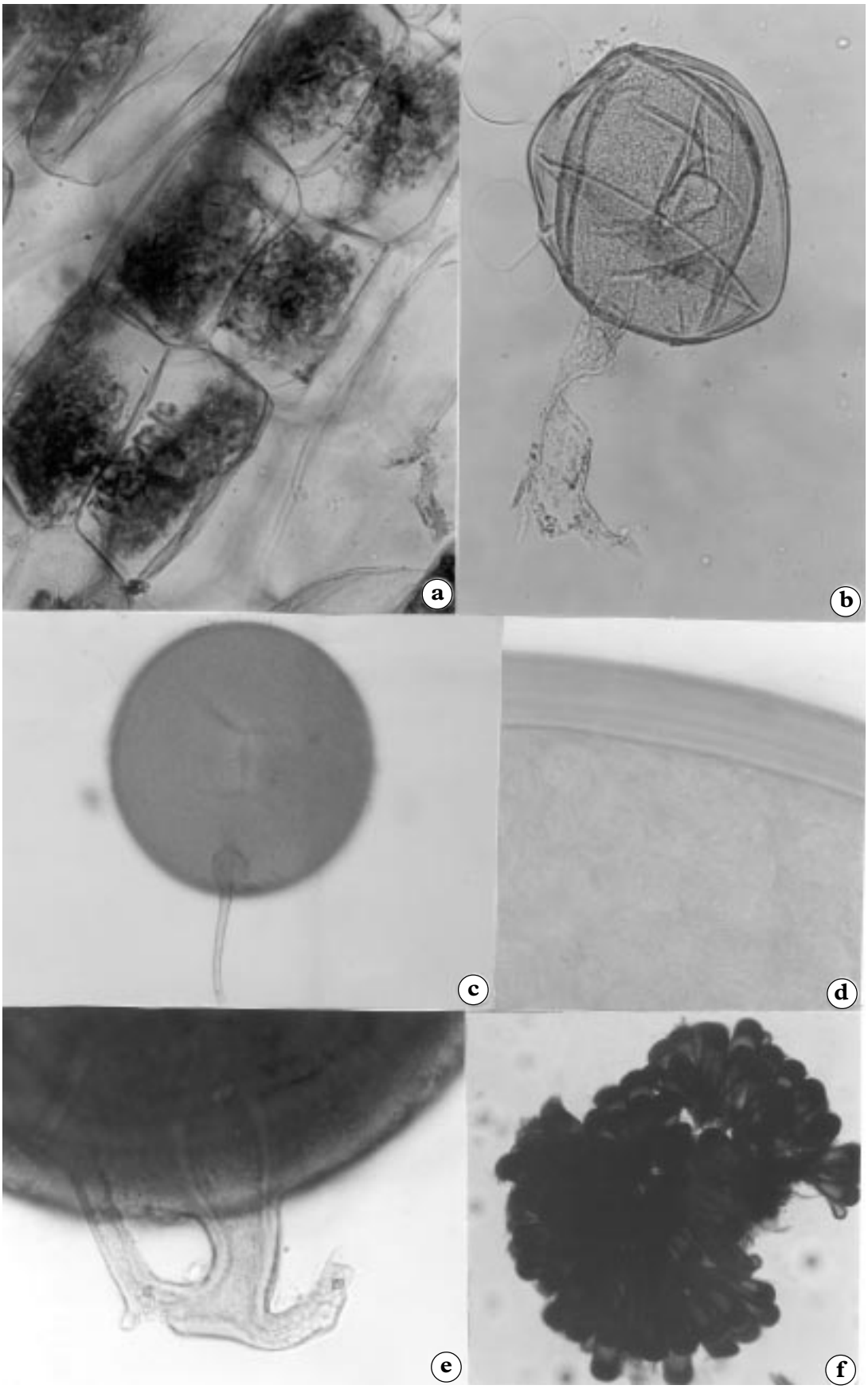

Plate 1

a. Arbuscular colonization of AM Fungi in *Gloriosa superba* L., (x 400).
b. Spore of *Acaulospora scrobiculata* Trappe, attached to degenerated saccule (x 200).
c. Intact spore of *Gigaspora margarita* Hall and Abott (x 100).
d. A portion of spore wall of *Gigaspora margarita* Hall and Abott showing wall lamination (x 400).
e. A portion of spore of *Glomus heterosporum* Smith and Schenck (x 400).
f. Crushed sporocarp of *Sclerocytis clavispora* Wu and Chen showing the spores with thick Apex.

Results and conclusion

In the present study, arbuscular mycorrhizal colonization was restricted to the epidermal and the cortical parenchymatous cells near the roots bases. The root exhibited fairly good colonization by AM fungi. The root colonization was characterized by the presence of hyphal, arbuscular (Plate 1) and vesicular colonization. The average root colonization of 50.38%, and a spore density of 250.6 spores per 100 g rhizosphere soil was recorded during the study.

A total of seven AM fungi belonging to four genera viz., *Acaulospora*, *Glomus*, *Gigaspora*, and *Sclerocystis* (Plate 1) were recorded from the rhizosphere soil of *Gloriosa superba* L. The recovered species were as follows: *Acaulospora scrobiculata* Trappe, *Gigaspora margarita* Hall and Abott, *Glomus fasciculatum* (Thaxter) Gerd. and Trappe emend. Walker and Koske, *Glomus geosporum* (Nicholson and Gerdemann) Walker, *Glomus heterosporum* Smith and Schenck, *Sclerocystis clavispora* Wu and Chen, and *Sclerocystis rubiformis* Wu and Chen.

The present study confirms the earlier findings (Rodrigues 1996; Bhat and Kaveriappa 1997) that AM fungi colonize tubers and extends the list of medicinal plants exhibiting AM fungal association.

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Glomus pansihalos: a new record from India

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Abstract

Glomus pansihalos was isolated from the rhizosphere soil of *Nerium oleander* L. It is a new record for India. The spores are globose-subglobose, dark brown, single, 102–148 x 101–145.8 µm, composite wall 6.5–12.5 µm, three walls in a single group, pore in the subtending hypha open.

Introduction

During a survey (2000/01) of rhizosphere soils of medicinal plants from Andhra Pradesh, spores of a hitherto unreported species of arbuscular mycorrhizal fungus, *Glomus pansihalos*, Berch, and Koske were isolated. The sieving and decanting method of Gerdemann and Nicholson (1963) was used for the isolation of spores. *G. pansihalos* was isolated from the rhizosphere soil of *Nerium oleander* Linn., Apocyanaceae. The species is reported for the first time from India (Bilgrami, Jamaludin, and Rizvi 1981, 1991; Sarbhoy, Varshney and Agarwal 1986; Sarbhoy, Agarwal, and Varshney 1996; Manoharachary, Kunwar, and Mukerji 2002).

Figure 1 *Glomus pansihalos*, Berch, and Koske

Spores globose-subglobose, 102–148 × 101–145.8 µm, dark brown, borne on a single subtending hypha, singly in soil. Wall of spore composed of a single group consisting of three inseparable wall layers. Wall 1 dark brown, 2.9–4.5 µm; wall 2 brown, smooth, laminate, 3–6.5 µm; wall 3 dark brown, 1–2 µm. Subtending hypha: straight, flared, constricted, 10.6–19.7 µm at the point of attachment, 12.4–15.5 µm below the point of attachment, brown-yellow, paler than spore wall, single, perpendicular to spore or recurved. Wall of subtending hypha composed of 1–3 wall layers, continuous with that of spores, 3.7–6.2 µm thick at the point of attachment, thinning distally, Wall 1 extending along subtending and vegetative hyphae, becoming thin and patchy. Pore in subtending hypha open.

Mycorrhizal associations: In the field associated with the rhizosphere soil of *Nerium oleander*, a medicinal and flowering plant.

Isolated by S Swarupa Rani on 6 June 2000 from Himayatnagar, Hyderabad, Andhra Pradesh. Specimens deposited in Botany Department, Osmania University, Hyderabad, (OUFH-168). A part of collection deposited in IARI, New Delhi, HCIO-44419.

The present isolate differs from Berch and Koske's (1986) in having darker (darker brown) spores and no warts in Wall 2.

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New approaches

Estimation of biomass of external mycelium of ectomycorrhizal fungi

In-growth mesh bags were used by Wallander, Nilsson, Hagerberg, and Baath (2001) to quantify the production of external mycelium of EM (ectomycorrhizal) fungi in the field (*New Phytologist* **151**(3): 753–760). Colonization of the mesh bags was followed by visual estimation of the amount of mycelium, and by measuring fungal biomarkers (the PLFA [phospholipid fatty acid] 18:2 omega 6, 9 and ergosterol). Mesh bags were placed inside and outside plots that were root-isolated in order to estimate the amount of saprotrophic mycelium in relation to EM mycelium. The majority of mycelium in the mesh bags was EM and this was confirmed by analysis of the delta C-13 value in mycelia. Fungal colonization of mesh bags peaked during autumn. The total amount of EM mycelium produced in the mesh bags during a year was calculated to be between 125 and 200 kg per ha. The total amount of EM mycelium (including EM mantles) in the humus was estimated to be 700–900 kg per ha. The biomass of EM mycelium in the soil was in the same range as the biomass of fine roots. Peaks of mycelial growth coincided with periods of maximum growth of fine roots.

Centre for Mycorrhizal Culture Collection

Collection and propagation of arbuscular mycorrhizal fungi

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In any branch of biological science, there is a fundamental need for accurate characteristics and classification of the organisms used in research. There are several issues, which must be considered when traditional taxonomic identification of spores is used to describe the community diversity of AMF (arbuscular mycorrhizal fungal). Firstly, the relative abundance of spores of a species may not reflect its functional importance or even its relative biomass contribution to the community as a whole, that is, the number of spores in the soil may not reflect the relative degree colonization of roots by this fungus or the amount and distribution of hyphae in the soil. Secondly, non-sporulating species may be present (Clapp, Young, Merryweather *et al.* 1995). A fungus may be a significant member of the 'vegetative' community, but, because of date of sampling, local environment, or host plant regulation of carbon expenditure, it may be unable to produce spores and yet be well able to persist to the following year as infective hyphae in roots or soil. Thirdly, spores collected from the field may be difficult to identify as a result of degradation of spore walls (Morton, Bentivenga, and Wheeler 1993).

Several approaches can be used to address these issues. Molecular methods can be used to detect taxa or taxonomic groupings of AMF in roots or soil. In addition, non-sporulating species can often be coaxed to sporulate in 'trap cultures'. Cultures of these fungi are also necessary to provide living fungal and mycorrhizal root material for research and practical applications. Trap cultures also provide healthy inocula to establish multispore or single-spore cultures of component species. Propagation of arbuscular mycorrhizae cultures requires their growth in association with a living plant.

Introduction of the pot culture technique of Mosse and Thompson (1984) has rendered the production of AM inoculum technically feasible. Generally pot cultures are raised by growing an inoculated host plant in a sandy soil supplemented with a nutrient solution with low levels of phosphorus (Menge 1984). But there are many other potting mixes used for trap cultures viz., expanded clay, peat, perlite, vermiculite, soilrite, water absorbing polymers, and a variety of other substrates. Cultures are monitored for sporulation by routine sampling. Spores produced in pot culture are in a

condition more conducive to identification than the original spores collected from Morton the field and, usually result in complex mixtures of species which change over time (Morton, Bentivenga, and Wheeler 1993; Stutz and Morton 1996; Brundrett, Jasper, and Ashwath 1999a). Changes in species richness in experimental microcosms can be used to indirectly measure partitioning effects of host and soil factors on niche occupation.

The CMR (Centre for Mycorrhizal Research), has the facility of conserving mycorrhizal germplasm. From each study site, three blocks are chosen and samples are taken from the rhizosphere of host plants at a depth of 0–30 cm, there are 5–9 samples from each block (depending upon the site's topography). The samples are air dried in shade to a point where there is no free moisture and are filled in plastic bags, sealed and stored at 4 °C in a cold room until further processing. A preliminary, thorough sampling of the study site is conducted: spore count, per cent root colonization, diversity, and infectivity potential of the cultures are measured, and trap cultures are initiated. The cultures are monitored regularly for spore production, root colonization, and the species richness of the site is then calculated.

Trap cultures are established in different-sized pots (500 ml, 1.5 litres, and 10 litres) using the whole inoculum (roots, infectious propagules, and surrounding material, that is, soil from a field sample. The roots are chopped into small fragments and mixed with the rhizosphere soil. This blend is mixed 1:1 (v/v) with autoclaved coarse sand (collected from the Sindh river of west Bundelkhand region, U P, and sieved through 30 mesh size BSS; [British Standard Size] aperture size 0.53 mm) or vermiculite or terragreen (American aluminium oxide, Oil Dry US Special, Type IIIR) and placed in pots. Pots are seeded with a variety of host plants: *Allium cepa* (onion), *Allium porrum* (leek), *Sorghum vulgare, Lycopersicon esculentum* (tomato), *Capsicum sp. Medicago sativa* (alfalfa), *Trifolium alexandrium* (barseem), *Daucus carota* (carrot), *and Tagetus sp.* (marigold).

For each field, three replicate pots are raised. Three pots of non-mycorrhizal controls are also raised. Five pregerminated seeds of each species are placed at different places. In the non-mycorrhizal pots, autoclaved soil (at 120 °C for 1 hour at 15 psi) is used in place of inoculum keeping all other conditions same. Cultures are grown in a greenhouse at 35 ± 5 °C at 60% relative humidity. The pots are watered at regular intervals to maintain the soil moisture content (gravimetrically) at approximately 60% of the water-holding capacity.

The pots are arranged on a greenhouse bench in a completely randomized design with three replications. Half-strength Hoagland's nutrient solution (Hoagland and Arnon 1938) is provided to the plants at fortnightly intervals. After four months of the growth cycle, the pots are left to dry undisturbed at a fairly stable temperature so that the drying is not too rapid. After completion of the growth cycle, the dried shoots are cut at ground level without disturbing the substrate and seeds of different hosts are sown. After each cycle, core sampling is done from the pots to study spore number, number of AMF species, per cent root colonization, and infectivity potential of AMF.

Using the successive trap culture technique; species richness, sporulation rate, per cent root colonization, and infectivity potential of AMF in germplasm was found to be comparable to that found in most plant communities. Direct field sampling indicated a richness of 1 to 6 species per sample. Comparable numbers of species were recovered from one cycle of trap cultures. However, 75% of the species richness measured after two or three propagation cycles was not detected in the first trap culture cycle. This indicates that additional cycles of pot cultures are required for fungi colonizing roots to begin to sporulating enough to be detected.

The host plants are known to affect the diversity of AMF in fields (Bever 2002). Many host plants are chosen as trap plants. Grasses and legumes are known hosts for successful propagation of AMF and hence are included as trap plants. *Sorghum* is used mainly because of its compatibility with many fungal species in all genera (except *Sclerocystis*) originating from a wide range of habitats (Morton, Bentivenga, and Wheeler 1993). Marigold is chosen as a trap plant as it gives a good root biomass and a good host for the propagation of AMF. Onion and subterranean clover (*Trifolium subterraneum*) are also used widely as culture hosts.

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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.

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- *Acta Societatis Botanicorum Poloniae*
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- *Applied Soil Ecology*
- *Arbuscular Mycorrhizae: Interactions in Plants, Rhizosphere and Soils*
- *Biotechnology of Biofertilizers*
- *Canadian Journal of Botany–Revue Canadienne de Botanique*
- *Chemosphere*
- *Communications in Soil Science and Plant Analysis*
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Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

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