Vol. 15 No. 1
April 2003

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 disturbed lands. Part 1. Effect of soil disturbance and tilling on mycorrhiza

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Mycorrhizal activity is maximum in surface soil up to a depth of 10–15 cm. Any kind of disturbance in this layer of soil may affect mycorrhizal activity by disrupting its hyphal network, which may delay or inhibit the infection of plant roots by mycorrhizal fungi. Developmental activities (road building, construction of dams, etc.), natural agencies (erosion, by rivers, rains, avalanches, storms), tilling, compacting, soil aggregation, mining, removal of organic matter from the forest floor and grazing may cause soil disturbances. This paper deals with the effect of soil disturbance and tilling on the development of mycorrhiza in plants.

In order to evaluate the impact of soil disturbance on mycorrhizal activities, soil in the green house or in the field is subjected to artificial disturbance before raising experimental plants. It is hypothesized that the disruption of the microstructure of a previously zero-tilled soil is capable of reducing the vesicular-arbuscular mycorrhizal infection to a degree that may affect the uptake of phosphorus.

Effect of soil disturbance on mycorrhiza

Effect of soil disturbance on mycorrhizal population and root infection

Studies conducted at the United States Department of Agriculture, Pacific North-West Research Station, Corvallis, USA, showed that following forest disturbance, survival of propagules and input of spores of ectomycorrhizal fungi appeared insufficient to maintain the mycorrhizal potential of disturbed sites in the absence of living hosts. Ectomycorrhizal formation in seedlings generally decreased as the length of time between disturbance and reforestation increased. The degree of organic matter lost from a site can influence populations of mycorrhizal fungi. Mycorrhizal formation was reduced by 90% and basal area by 43% in Douglas fir (*Pseudotsuga menziesii)* seedlings grown in soils where forest practices drastically reduced organic matter levels (Amaranthus and Perry 1990).

Studies were conducted at the Centro de Ecologia Y Ciencias Ambientales, Instituto Venzolano de Investigaciones, Cientificas, Apartado, Caracas, Venezuela, to investigate recolonization, by VAM propagules, of Savannah severely disturbed by removing the soil organic layers with bulldozers for road building. The substrate was fertilized with 300 kg per ha of N:P:K (12:24:12) and limed (800 kg per ha). Introduced species, *Brachiaria decumbens, B. humidicola, Pueraria phaseoloides*, and *Calopogonium* sp. were sown on the site. The results showed that perturbation diminished the spore number from 200 spores per 100 g of soil in the undisturbed Savannah, to 0–20 spores per 100 g of soil at the disturbed site. In revegetated areas where *B. decumbens* and *B. humidicola* were grown, there was an increase in spore number (271–3076 spores per 100 g of soil) and VAM infection (64%–92%) (Cuenca and Lovera 1990).

In studies conducted at the Kamanaskis Centre for Experimental Research, University of Calgary, Calgary, Alberta, Canada, naturally regenerated seedlings of spruce (*Picea glauca* and *P. engelmanii*) were collected from undisturbed areas (mature forests), from sites disturbed by natural events (avalanche slopes, stream banks) and sites

disturbed by man (road cuts etc.). Seedlings were generally 1–6 years old, less than 7 cm tall and soil pH between 7.5–8.5 and occasionally, 4.0. Mycorrhizal inoculum potential was low in road cuts, moderate in clear cuts, and high in areas where seedling roots were in contact with the roots of mature trees. Mycorrhizal infections in seedlings growing on severely disturbed sites and in the absence of roots from other ectomycorrhizal hosts were dominated by E-strain fungi (*Complexipes, Wilcoxina), Mycelium radices-atrovirens, Amphinema byssoides, Tomentella* spp., non-agarics forming 72 per cent mycorrhizae. On gravel bars, E-strain, *Tomentella* sp. and *A. byssoides* accounted for 42 per cent of mycorrhizae. *Cenococcum geophilum* was also common. Willows and poplar roots were likely in contact with roots of many spruce seedlings. Seedlings regenerating under a canopy of spruce or lodge pole pine on avalanche slopes rarely bore the E-strain while *C. geophilum, Lactarius, Tricholoma, Cortinarius*, and *Piloderma* formed a significant fraction of mycorrhizae. *A. byssoides* and *Tomentella* spp. were also present. Mycorrhizal infections on seedlings growing on rotten wood were dominated by unknown basidiomycetes and *A. byssoides*. Late stage fungi occurred on seedlings when they were exposed to inocula from roots of mature trees (Danielson and Pruden 1990).

In studies conducted at the Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Australia, plant bioassays were used to compare the infectivity of VA-mycorrhizal fungi in three soils from different vegetation types, before and after disturbance. If soil from annual pasture was disturbed, VAM infectivity was not decreased. By contrast, in soils from native vegetation, the percentage root length colonization was halved. The pasture soil had up to 25 times more disturbance tolerant propagules than the soil from native vegetation (Jasper, Abbott, and Robson 1990).

In studies conducted at the Department of Forestry, North-Eastern Regional Institute of Science and Technology, Nirjuli, India, maximum VAM populations were recorded in undegraded forest sites, and lowest VAM populations were found in highly degraded sites (Singh and Tiwari 1999).

In studies conducted at the Slippery Rock State College, Slippery Rock, PA, USA, twelve, 0.1 ha disturbed plots were established. The treatments were: top soil removed; subsoil removed; spring ploughed; spring ploughed and disced; spring ploughed, disced and VAM added; spring ploughed, disced and pramitol added; spring ploughed, disced and planted with corn; spring ploughed, disced, atrex added and planted with corn; spring ploughed, disced and fertilized with 10:10:10 NPK; fall ploughed; fall ploughed and disced; and burned. Disturbed plots were allowed to undergo natural succession. After one growing season after abandonment, 43 plant species representing 19 families were found and 42 species had mycorrhiza. Mycorrhizal infection ranged from 5%–91%. After three growing seasons, 48 species representing 19 families were found and 46 had VA mycorrhizae. Mycorrhizal infection ranged from 5%–95% (Medve 1981).

Studies conducted at the Department of Botany, North-Eastern Hill University, Shillong, India, showed that the plant species growing in more degraded soil (slash burn agriculture on a five-year cycle) had higher mycorrhizal infection than those in a less disturbed site. Maximum spores were counted in winter months. No marked seasonality was observed in mycorrhizal infection. Fewer endogonaceous spores were present in the soil of the less disturbed site (Kshatriya, Jha, Sharma, *et al.* 1990).

Effect of soil disturbance on hyphal network of mycorrhizal fungi

In studies conducted at the University of Western Australia, School of Agriculture, Nedlands, Australia, a nylon mesh was used to exclude plant roots, and allow fungal hyphae to grow into the soil contained by the mesh in two glasshouse experiments to determine the effect of soil disturbance on the infectivity of external hyphae of VA-mycorrhizal fungi. Hyphae of the VA-mycorrhizal fungi that have been separated from the original host plant roots were still able to colonize bioassay plants rapidly and extensively even if the soil was dried to -2.1 MPa prior to the separation. However, disturbance of the soil inside the mesh, by mixing for one minute, almost eliminated subsequent VA-mycorrhizal formation in that soil. This damage to soil hyphal networks by soil disturbance may contribute to the losses in mycorrhizal infectivity that have been observed after mining and cultivation (Jasper, Abbott and Robson 1989a, 1990).

In studies conducted at the Department of Land Resource Science, University of Guelph, Guelph, Ontario, Canada, the mechanism by which VAM colonization of young maize plants was inhibited by soil disturbance was examined. Plastic cylinders divided into three compartments by a fine nylon mesh, impervious to roots but not to mycorrhizal fungal hyphae, were filled with soil and maize plants were grown in two outer cylinders over a series of three-week cycles. Prior to the final growth cycle, the root-free soil in the central compartment was either disturbed and repacked or left undisturbed. In another treatment, the hyphal connections between the outer and central sectors were severed while leaving the soil in all sections undisturbed. Plants were then grown in the central compartment. Mycorrhizal colonization and shoot P and Zn concentrations of the plants grown in the root free zones were substantially reduced by the

disturbance of the soil. The reduction in colonization was similar to that found in control pots in which the root system and mycelial networks were disturbed. This suggested that the destruction of the hyphal network was an important component of the disturbance phenomenon. Removal of hyphal connections with roots in the outer compartments had no effect on colonization or nutrient absorption. Isolation of the mycelial network from the root system from which it had originated did not then seem to affect its infectivity (Evans and Miller 1990).

In experiments conducted at the School of Biological Sciences, University of Sydney, Australia, soil was collected from a natural pasture adjacent to a recently prepared cotton field and sieved through a 2 mm grid. During examination of the types of fungal propagules initiating infection of plant roots after disturbance, it was observed that the density of spores was moderate. Though spores are probably an important reservoir of fungal propagules, results indicated that hyphal fragments in soil might initiate some infection following severe disturbance (Heath and McGee 1992).

In studies conducted at the Pennsylvania State University College of Agricultural Sciences, Department of Horticulture, University Park, Pennsylvania, USA, soil filled pouches which either allowed root entry or excluded roots were buried in the root zone of field grown corn (*Zea mays*). Following the harvest in the fall, the pouches were either removed undisturbed or were disturbed outside the patches or were disturbed both inside and outside the pouches. Total and metabolically active AM hyphae in the undisturbed pouches declined by 20% and 33%, respectively (average of coarse and fine mesh treatments) from fall to spring presumably because of over-winter death. In spring, living hyphae were more abundant in the presence of roots than in their absence. Total hyphal density, the metabolically active hyphal density and the proportion of total living hyphae progressively diminished with increased disturbance. Over-winter survival of arbuscular mycorrhizal fungi was favoured by attachment of roots but diminished by disturbance (Kabir, O' Halloran, and Hamel 1997).

In further studies conducted at the above University, AM fungal infection was established on maize (*Zea mays*) plants grown in pots (divided into two compartments by a 37 mm nylon mesh) for six weeks in an unsterilized field soil. The mesh prevented plant roots but allowed fungal hyphae to grow from one side of the pot to the other. After AM establishment, the soil was either disturbed by sieving through a 2 mm mesh (D) or undisturbed (U) leading to four combined disturbance treatments. These treatments were: 1, both compartments undisturbed (UU); 2, root compartment disturbed and root free compartment undisturbed (DU); 3, root compartment undisturbed and root free compartment disturbed (UD), and 4, both compartments disturbed (DD). The effects of fallows of four different durations of 0, 30, 60, and 90 days were also measured in the same experiment giving a total of 16 treatments. The soil was disturbed at the beginning of the experiment in the root compartment and after each fallow period in the root-free compartment. Immediately after disturbance of the soil in the root free compartment, corn was planted and grown for 30 days. Soil disturbance had no adverse effect on AM efficiency if test plants were planted immediately after disturbing the root free compartment. However, AM efficiency decreased with increasing length of fallow. Lengths of total and metabolically active extraradical hyphae in the root free compartments were measured before each fallow. Significantly less hyphal lengths were observed in pots where soil of the root compartment had been disturbed. Root weights of test plants were highest in UU and lowest in DD treated plants. The phosphorus content of the test plants was twice as high in UU as in DD treatments. Test plants in UU pots had greater Zn and Cu contents than DU, UD or DD pots. Contents of P, Zn, and Cu in the test plants were reduced by about 40%, 63%, and 70%, respectively, by 90 days of fallow when compared to 0 days of fallow (control) (Kabir, O'Halloran, and Hamel 1999).

Effect of soil disturbance on nutrient uptake, plant growth and mycorrhizal infection

In studies conducted at the Department of Land Resource Science, University of Guelph, Guelph, Ontario, Canada, undisturbed soil cores (within plastic cylinders) were excavated from long term zero-tilled plots. Maize (*Zea mays*), wheat (*Triticum aestivum*), spinach (*Spinacea abraces*) or rape (*Brassica napus*) was grown in the cores after being subjected to structurally disruptive forces and then packing them into identical cylinders. Soil disturbance significantly reduced phosphorus and zinc absorption and mycorrhizal infection in maize and wheat roots (both mycorrhizal species) in soils originating from three sites differing in local geography and/or texture. This effect was not found in spinach or rape roots (both non-mycorrhizal species). Injection of benomyl, a potent inhibitor of mycorrhizal fungi, into soil surface significantly reduced the influence of soil disturbance upon phosphorus absorption. No significant differences were found in VA mycorrhizal infection within fungicide treated cores (Evans and Miller 1988).

In further studies conducted at the above University, maize was grown for three-week cycles on disturbed soil. At the conclusion of each cycle, half the pots were disturbed and replanted while the

other half were replanted without disturbance. There was a concurrent and rapid increase in VA mycorrhizal infection and phosphorus absorption by maize over three growth cycles when an initially disturbed soil was kept undisturbed. This provides good evidence that soil disturbance reduces VA mycorrhizal infection and phosphorus absorption by maize (Fairchild and Miller 1988).

In further studies conducted at the above University, maize was grown for four three-week cycles in pots containing soils, which were either unpasteurized or pasteurized or pasteurized and inoculated with *Glomus* sp. The soil was either disturbed by hand (D) or left undisturbed (U) after each of the first three growth cycles. At the end of the final growth cycle, shoot dry mass and shoot phosphorus concentrations were higher in plants in the U soils relative to the D soils, by a factor of 1.5 and 1.9, respectively, with a shoot phosphorus concentration of 3.81 mg per g for plants in the U soil in the unpasteurized treatment. In the pasteurized soil, there was no difference in either shoot dry mass or shoot phosphorus concentration between the plants in the U and D treatments and shoot phosphorus concentration in the pasteurized soil was indicative of strong phosphorus deficiency (a mean of 1.24 mg per g of phosphorus). In the inoculated soil, shoot dry mass was similar in the two disturbance treatments while shoot phosphorus concentration was significantly higher (by a factor of 1.2 at 2.4 mg per g of phosphorus) in U treatment compared to 1.83 mg per g in the D treatment. In the unpasteurized soil, AC (arbuscular colonization) was strongly increased in the U compared to D soil. No arbuscules were present in the roots of pasteurized soil. In the inoculated soil, AC was moderate to high but the effect of disturbance on AC was inconsistent from one replicate to the other. Pasteurization eliminated the mycorrhizal fungi and at the same time removed the enhancing effect of the shoot phosphorus, leaving plants in both U and D treatments deficient in phosphorus. This result along with the higher phosphorus absorption and mycorrhizal colonization in the U, relative to the D treatment in the unpasteurized soil, and along with the return of a disturbance effect following the reintroduction of mycorrhizal fungi in the inoculated soil, strengthens the conclusion that mycorrhiza are indeed the means by which lack of disturbance promotes phosphorus absorption (McGonigle and Miller 1992).

In experiments conducted at the School of Biological Sciences, University of Sydney, Australia, soil was collected from a natural pasture adjacent to a recently prepared cotton field and sieved through a 2 mm grid. Development of mycorrhizae over 42 days in cotton seedlings grown in the sieved soil was delayed and reduced when compared to seedlings in the undisturbed block of soil (Heath and McGee 1992).

Impact of amendment of disturbed soil with phosphorus on plant growth and mycorrhizal development

In studies conducted at the Department of Land Resource Science, University of Guelph, Guelph, Ontario, Canada, a growth room experiment was conducted where maize was grown for three-week cycles in initially disturbed soil with which phosphorus was mixed to create five phosphorus amendments at 0, 40, 80, 120, and 160 μg P per g of soil. After each growth cycle, the soil in half the pots was disturbed and replanted whereas the other half was replanted without disturbing the soil. In the third growth cycle, shoot dry weights were greater in the undisturbed soil than in the disturbed soil at the lower phosphorus amendment rates but soil disturbance had no effect at the 120 and 160 μg P per g of soil amendments. Mycorrhizal colonization was much greater in the undisturbed soil at all P amendments rates. Shoot N, P, Mg, and Zn concentrations were higher in the undisturbed than in the disturbed soil at all P amendments but only the increased P and Zn could be directly related to increased mycorrhizal colonization. While the benefit to the plant from the mycorrhizal colonization induced by lack of soil disturbance can be quite large, even at the high rates of P amendment to the soil, the benefit achieved depends on the balance between the carbon cost and P benefit to the host and this may or may not favour the host (Fairchild and Miller 1990).

Effect of timing and frequency of disturbance on mycorrhiza

In studies conducted at the Department of Land Resource Science, University of Guelph, Ontario, Canada, paired disturbed and undisturbed cores were collected from a no-till soybean (*Glycine max*) field on 16 July, 1991 (when the soybean crop was in a vegetative stage), on 8 October (shortly after harvest), on 14 November (shortly before soil freezing), in April the next year (shortly after thawing) and on 6 May (prior to planting the subsequent crop). Maize was grown for three weeks in the disturbed and undisturbed cores following field collection. Shoot dry mass and P concentration were greater for plants growing in the undisturbed than in the disturbed soil cores at all sampling times. The shoot P content of the plants in the undisturbed soil was 2.6, 1.9, 2.6, 1.6, and 1.8 times the P content in the disturbed cores collected in July, October, November, April, and May, respectively. Arbuscular colonization of the roots in the undisturbed soil was 1.5 and 1.1 times that in the disturbed soil in July and October, respectively. There was however no difference in AM colonization in undisturbed and disturbed soils in

November and May samplings. Arbuscular colonization in the July sampling was lower than that in other sampling periods. There was no apparent relationship between hyphal length density or viable spore number and colonization. The results indicated that the effect of soil disturbance on phosphorus absorption was not related to the season in which the soil was collected but there was an effect on arbuscular colonization. The infectivity of the propagules appeared to increase from July to October but remained constant through the fall and retained similar infectivity the following May (Miller, Mitchell, Pararajasingham, *et al*. 1992).

In further studies conducted at the above University, maize (*Zea mays*) was grown for four three-week cycles in pots, which initially contained disturbed soil. Five soil disturbance treatments were used to assess the impact of the frequency with which the soil is disturbed and the impact of timing of disturbance on mycorrhiza. The frequency of soil disturbance had a major effect on mycorrhizal colonization while the timing of soil disturbance was more related to the reduction in shoot P absorption resulting from the disturbance. The results suggest that the extra radical mycelium plays a key role in the mechanism by which soil disturbance reduces shoot P absorption (McGonigle and Miller 1993).

Effect of degree of soil disturbance on mycorrhiza

Growth chamber experiments on maize, conducted at the Department of Land Resource Science, University of Guelph, Ontario, Canada, showed that cutting the soil into 1, 2 or 4 cm cubes reduced P absorption and shoot dry matter but the reduction was not as great as that after hand disturbance of the soil to the extent that it could pass a 5 mm sieve (McGonigle, Evans, and Miller 1990).

In studies conducted at the Department of Conservation and Land Management, Research Division, State Operation Headquarters, Australia, intact soil blocks (20 x 20 x 15 cm) were taken from each of two sites on sandstone soils and the blocks were cut longitudinally into four (dist. 1), nine (dist. 2) and twenty five (dist. 3) equal portions. Seeds of *Trifolium repens* were sown in the blocks and the plants were harvested 14, 21, 28, 35, and 42 days after sowing with total root length, root length colonized by VAM, and shoot dry mass measured at each harvest. VAM colonization was found to have commenced by 14 days in the roots of seedlings grown in dist. 1 and dist. 2 soil blocks but the initiation of this VAM colonization was delayed by up to six weeks for seedlings grown in dist. 3 soil blocks. The low (dist. 1) and the intermediate (dist. 2) degree of soil disturbance did not cause a delay in the initiation of VAM infection but did significantly reduce the proportion of root length colonized by VAM fungi after 21 days. Shoot dry mass was significantly lower in the seedlings grown in dist. 3 soil blocks but not in low and intermediate soil disturbance treatments after 21 days. It is thus concluded that the most severe experimental disturbance probably disturbed the external hyphal network and root fragments (containing hyphae and vesicles), which in turn temporarily reduced the infective potential of the fungus to nil. The observed delay in the initiation of VAM in the most disturbed blocks can, therefore, be explained by the time required for hyphae to grow from the propagules in the soil, which remained viable after the disturbance (Bellgard 1992b, 1993).

In experiments conducted at the University of York, Department of Biology, Yorkshire, England, colonies of bulbs of *Hyacinthoides non-scripta* were removed and replanted during the rootless phase in summer after the soil around and below the bulb had been mixed (major disturbance) so as to disturb the external mycelium of AM fungi. As a minor disturbance treatment, topsoil was removed; bulbs were turned or not turned in their growth position with as little other disturbance as possible and the topsoil was replaced. Control plants were left undisturbed. Half of the plants were harvested 3–4 weeks after the onset of root emergence. Populations of AM fungi in roots were greatly reduced by major disturbance while those in other treatments and control were unaffected. At the second harvest, in spring, when shoots had emerged, root colonization by fine endophytes and *Scutellospora* morphotypes developed in all treatments while that of *Acaulospora* morphotypes remained low after the major disturbance. At the second harvest, disturbance treatments delayed the appearance of mycorrhizas with degenerate arbuscules. Leaf phosphorus concentration was unaffected by soil disturbance, possibly due to partial recovery of AM fungal populations or buffering by resources stored in the bulb (Merryweather and Fitter 1998).

Effect of tilling on mycorrhiza

Studies were conducted at the Pennsylvania State University, Department of Horticulture, USA, on the influence of tillage practices on native VAM fungi in two consecutive years in two 11-year-old, long term, tillage-fertilizer experimental field soils (a sandy loam and a clay) growing corn (*Zea mays*) in monoculture. The three tillage practices were: 1, CT (conventional tillage) (fall ploughing plus spring discing); 2, RT (reduced tillage)(spring discing) and 3, NT (no till). The corn crop received either inorganic N and K or liquid dairy manure. Densities of total and metabolically active VAM hyphae in soil and mycorrhizal root colonization at the 12–14 leaf stage were significantly lower in CT soil than in RT and NT soils. The lowest soil hyphal densities were observed in early spring.

The levels of intra- and extra radical fungal colonization always increased from spring to silking and decreased thereafter. Spring discing had only a small and transient negative effect on hyphal abundance in soil. Fertilization did not influence mycorrhizal colonization of corn and abundance of soil hyphae in sandy loam soil but in clay soil, metabolically active hyphae were more abundant with manure application than with mineral fertilization (Kabir, O'Halloran, Fyles, *et al.* 1997).

In further observations from experiments conducted at the above University, soil samples collected from the plant row, 18.75 cm from the row (quarter of inter-row distance) or from in-between rows (37.5 cm from the row) at the 12–14 leaf stage, silking stage and at harvest showed that the density of total and viable AM hyphae were greatest in the row, lowest in mid-row and increased steeply from the 12–14 leaf stage to the silking stage and decreased thereafter. Hyphal densities were higher in NT soil than in CT soil while RT soil contained intermediate hyphal densities. The highest corn P, Zn, and Cu concentrations were observed in NT and RT treatments while concentrations of K, Ca, and Mg did not change with tillage or fertilization type. Manuring significantly increased densities of total and viable hyphae in clay soil (Kabir, O'Halloran, Fyles, *et al.* 1997).

Further studies conducted at the above University showed that root colonization, total hyphal density and spore density were correlated and were highest at a depth of 0–15 cm in soil. Tillage significantly reduced total hyphal density and spore density at 0–5 cm depth, but did not affect root colonization. Ploughing below 15 cm is likely to diminish AM fungal inocula in the rooting zone of establishing seedlings (Kabir, O'Halloran, Widden, *et al.* 1998).

A field trial was established at the Queensland Wheat Research Institute, Toowoomba, Australia, on a vertisol that had been clean fallowed for eight years. The design comprised a factorial of three tillage (zero, reduced and frequent) × two stubble (burnt or retained) × four fertilizer treatments (nil, 40 kg P per ha, 15 kg Zn per ha or Zn + P). The chemical characteristics of the soil were: 22 mg per kg bicarbonate extractable P, 0.27 mg per kg DTPA extractable Zn, pH 8.2 at a soil depth of 0–15 cm. Two successive wheat crops were grown on the long fallow site. VAM colonization potential was bio-assayed by taking intact soil cores to a depth of 15 cm in ten cm diameter PVC tubes. A test crop of linseed (*Linus usitatissimum*) was sown in these cores and VAM colonization of the roots was determined at depths of 0–5, 5–10 and 10–15 cm after 4 and 8 weeks growth. Linseed plants at four weeks had only 8 per cent VAM colonization in cores taken before the first wheat crop compared with 32% before the second wheat crop. The second wheat crop had 24% VAM colonization of roots at 11 weeks and a grain yield of 1.0 tonnes per hectare while the wheat grown in the adjacent long fallow soil had 2 per cent VAM colonization and a grain yield of 0.6 tonnes per hectare. At a soil depth of 5–10 cm, the per centage of VAM decreased as the number of tillage operations increased. However, at soil depths of 0–5 and 10–15 cm, the percentage of VAM increased with tillage. Linseed growth was similar between tillage treatments, especially in stubble burnt treatments (Peck, Thompson, and Haak 1992).

Studies were conducted at the University of Guelph, Department of Land Resource Science, Guelph, Canada, on maize (*Zea mays*) grown in plots with previous crops of maize and *Brassica napus-canola*, with no tillage or with conventional tillage and with or without phosphorus fertilization (five levels). No tillage resulted in high shoot P concentrations but lower shoot weight than conventional tillage. Greater shoot P in no-tillage treatments was related to rapid intra-radical development of mycorrhiza (with maize as the previous crop) or rapid connection to a mycorrhizal mycelial network. Maize yield and harvest index were lower after cropping with canola. The yield of maize with conventional tillage was higher than that with no tillage but the harvest index was lower (Gaveto and Miller 1998).

Studies were conducted at the United States Department of Agriculture, SE-Agricultural Research, Lincoln, Nebraska, USA, to determine the effect of tillage and nitrogen fertilization on mycorrhizal infection of winter wheat (*Triticum aestivum*). The treatments consisted of plough, subtill and classical fallow, split into 0 and 44 kg nitrogen per hectare, application. Increased tillage and nitrogen fertilization decreased mycorrhizal infection. However, chlorophyll content in the flag leaf and phosphorus concentration in the upper 15 cm soil were inversely related to infection rate within tillage treatments. Diffusive resistance tended to decrease with tillage (plough > subtill > classical fallow) but the reverse trend was found with transpiration. Grain yield did not appear to be correlated with mycorrhizal infection from the various tillage treatments but within tillage treatments, nitrogen fertilization decreased grain yield and mycorrhizal infection rate. There was a shift in mycorrhizal species with tillage treatment (Ellis 1981).

Survival of mycorrhizal fungi in stored top soil

Studies were conducted at the Department of Biology, University of Wollongong, New South Wales, Australia, on the infectivity of different types of VAM propagules in stored soil from an open woodland soil of low fertility, wet sieved through 1 mm, 250 μm and 106 μm sieves. Various fractions extracted were: 1, root fragment; 2, fungal hyphae and 3, VAM spores. Each fraction was tested to

determine its potential to initiate VAM association with plants recolonizing the disturbed sites. Hyphae of VAM fungi grew from root fragments within 14 days. VAM spore fraction initiated VAM infection after 28 days. VAM hyphal fragments did not produce any VAM infection even after 42 days (Bellgard 1992a).

Studies conducted at the Department of Biological Sciences, Stanford University, Stanford, California, on revegetation of stockpiled serpentine substrate showed that seed mixture applied to both subsoil and topsoil plots was largely ineffective for revegetation. Most of the growth of topsoil was from indigenous seeds. Phosphate application (100 kg P per ha as NaH_2PO_4) to the top soil plots resulted in a significant increase in total aboveground productivity attributed mostly to annual legumes (mostly *Lotus subpinnatus*) and to a lesser degree, *Plantago erecta*. Mycorrhizal root colonization for *P. erecta* was not affected significantly by phosphate application but was lower in this disturbed serpentine soil compared to other undisturbed serpentine annual grassland sites nearby (Koide and Mooney 1987).

Studies conducted at the Illinois State University, Normal, USA, on the storage of topsoil showed that a higher percent mycorrhiza was formed from undisturbed soil than from stored topsoil. The studies also showed that root fragments originating from undisturbed soil were more effective as a source of inoculum (using corn as test plants) than the fragments originating from stored soil. Also, higher infection of corn was found in experiments using 100 fragments than those using 50 fragments. The results showed that topsoil storage for three years had a detrimental effect on VA mycorrhizal populations in general and mycorrhizal fragments in particular (Rives, Bajwa, and Liberta 1979).

Studies conducted at the Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Australia, showed that during bauxite mining in *Eucalyptus marginata* forests, the infectivity of the propagules of VAM fungi in the soil was destroyed even when the soil was stripped and spread promptly without stock piling. 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 the 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 loss of infectivity during mining was not a seasonal response (Jasper, Abbot, and Robson 1989b).

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Research findings

Reaction of kashini (*Cichorium intybus* L.) to different native arbuscular mycorrhizal fungi

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The role of AM (arbuscular mycorrhizae) in enhancing the plant growth and yield of many plantation and field crops has been well documented (Mallesha and Bagyaraj 1995; Mallesha, Padmavathy, and Bagyaraj 1994). However, reports on their role in essential medicinal plants are very meagre (Gupta and Janardhanan 1991). Kashini (*Cichorium intybus* L.) is an important essential medicinal plant commercially cultivated in Vellore district of Tamil Nadu, India. The cultivated plant is used in Indian medicine as a tonic. The roots and leaves are used for the treatment of stomachic, diuretic, fevers, vomiting, for enriching and purifying the blood and pain in joints. The leaves are used as vegetable greens which are rich in vitamin C (0.13 mg/g), vitamin B (0.6 mg/g) and protein (2.18 mg/g). The present study was undertaken to select an efficient AM fungus for inoculating kashini.

Materials and methods

kashini plants growing in the experimental farm of AVVM Sri Pushpam College, Poondi (India) were collected along with an almost complete root system and surrounding soil. The roots were carefully washed, cleared with 10% KOH, stained with trypan blue (Phillips and Hayman 1970), squashed

under a coverslip and observed under a light microscope. The percent root colonization was determined based on the number of root segments colonized by AM fungi. AM spores were isolated by the wet-sieving and decanting method (Gerdemann and Nicholson 1963). The number of AM propagules present in the soil were calculated to 100 g dry weight of soil. The species level identification of different native AM fungi was done following the keys provided by Trappe (1982) and Schenck and Perez (1990).

Pot cultures of the different native AM fungi with onion (*Allium cepa* L.) were made by using the funnel technique of Menge and Timmer (1982). After four months, these pot cultures were used as a source of inoculum. Seedlings of kashini were raised on seeds beds of size 30 × 15 × 10 cm using a sterile sand-soil (1:1) mix. There were eight beds for kashini. For each bed different AM inocula (sand-soil based inoculum maintained with onion) containing spores, infected root bits and mycelial bits was added at the rate of 250 g inoculum. Four-week old seedlings were transplanted to pots containing 5 kg of sterile sand-soil (1:1) mix. The soil used in this study was an alfisol fine Kaolinitic, isohyperthermic, Typic Kanhaplustalfs. The potting mix had a pH of 7.4 and 2.4 mg of available P g^{-1} (NH_4F + HCl extractable) and an indigenous mycorrhizal population of 300 infective propagules per gram of substrate. In this experiment eight different native AM fungi (as in Table 1) maintained as pot culture on onion (*Allium cepa* L.) were screened against kashini for symbiotic response. Root pieces and soil mixture from onion separately with eight test fungi were used as mycorrhizal inocula. Inocula at the rate of 12,500 infective propagules per planting point based on the most probable number method (Porter 1979) was added at the time of transplanting. Two seedlings were maintained per pot. Six replicates were maintained for each treatment. Pots were maintained in a greenhouse. One set of plants without inoculation served as the control. The plants received 50 ml per plot of Ruakura nutrient solution (Smith, Johnson, and Cornforth 1983) without P, once in 30 days. The treatments were arranged following a randomized block design.

Eighty days after transplanting, plants were harvested for determining their mycorrhizal status, and growth response. The plants samples were oven dried at 60°C to a constant weight to get plant biomass (shoot + root). Number of leaves, width, and length of leaves were also recorded. The phosphorus and potassium content of the plants was determined after tri-acid digestion (Jackson 1973). The roots were assessed for AM infection by the grid-line intersect method (Giovannetti and Mosse 1980) after clearing the roots with 10% KOH and staining with trypan blue (Phillips and Hayman 1970). The root systems were carefully removed from the potting medium, washed and measured for root volume by the water displacement method. The data were statistically analyzed and the treatment means separated by Duncan's multiple range test (Little and Hills 1978).

Results and discussion

Microscopic examination of the roots of kashini plants growing in the experimental farm, showed intensive AM development from June to October, when these plants were in their active growth period. Intramatrical vesicles, arbuscules, mycelial coils, and spores were observed in the cortical tissue of the roots. Microscopic examination of their

Table 1 Effect of different native AM fungi on growth response of kashini plants after eighty days of transplanting (mean of five replicates).

	Growth response						
	Plant height		*Dry weight*				
Treatment	*Root* (cm/plant)	*Shoot* (cm/plant)	*Root* (mg/plant)	*Shoot* (mg/plant)	*Number of leaves/plant*	*Leaf length* (cm)	*Leaf width* (cm)
Uninoculated	9.3^{c}	24.0^{c}	880^{c}	5110^{c}	11^{c}	22.4^{c}	3.2^{c}
Acaulospora scrobiculata	14.6^{b}	28.0^{b}	1485^{ab}	7240^{ab}	15^{b}	27.4^{ab}	3.8^{b}
Gigaspora margarita	17.5^{a}	32.0^{a}	1680^{a}	8425^{a}	18^{a}	31.5^{a}	4.2^{a}
Glomus aggregatum	17.0^{a}	30.0^{a}	1640^{a}	7865^{a}	17^{a}	29.5^{a}	4.0^{a}
Glomus geosporum	14.8^{b}	28.2^{a}	1425^{ab}	7140^{ab}	16^{ab}	28.6^{ab}	3.9^{ab}
Glomus macrocarpum	15.2^{ab}	29.4^{ab}	1520^{b}	7620^{b}	16^{ab}	28.4^{ab}	3.8^{b}
Glomus mosseae	14.4^{ab}	29.0^{ab}	1514^{b}	7610^{b}	16^{ab}	29.6^{ab}	3.9^{ab}
Sclerocystis pakistanica	14.7^{b}	28.1^{b}	1430^{ab}	7125^{b}	14^{bc}	27.5^{ab}	3.7^{ab}
Scutellospora calospora	15.0^{ab}	29.4^{ab}	1532^{b}	7710^{ab}	15^{b}	28.0^{ab}	3.6^{b}

Means in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

Table 2 Effect of different native AM fungi on root volume, mycorrhizal colonization, shoot and P and K content of kashini plants after eighty days of transplanting (means of five replicates)

Treatment	*Root volume/ plant* (cm^3)	*Mycorrihizal colonization* (%)	*P content*		*K content*	
			Shoot (g/plant)	*Root* (g/plant)	*Shoot* (g/plant)	*Root* (g/plant)
Uninoculated	10.0[c]	90.0[a]	0.204[b]	0.0065[c]	0.262[c]	0.0044[c]
Acaulospora scrobiculata	16.0[b]	45.0[c]	0.224[ab]	0.0108[b]	0.302[a]	0.0064[a]
Gigaspora margarita	20.5[a]	27.0[c]	0.257[a]	0.0124[a]	0.302[a]	0.0055[a]
Glomus aggregatum	19.0[a]	85.0[a]	0.250[a]	0.0120[a]	0.280[b]	0.0065[a]
Glomus geosporum	14.0[c]	62.0[b]	0.226[ab]	0.0070[c]	0.272[bc]	0.0056[a]
Glomus macrocarpum	17.0[b]	66.0[b]	0.230[ab]	0.0077[ab]	0.282[b]	0.0062[a]
Glomus mosseae	16.0[b]	82.0[a]	0.228[ab]	0.0090[ab]	0.280[b]	0.0064[a]
Sclerocystis pakistanica	14.0[c]	63.0[b]	0.230[ab]	0.0092[ab]	0.302[a]	0.0048[c]
Scutellospora calospora	15.0[c]	45.0[bc]	0.233[ab]	0.0086[ab]	0.301[a]	0.0054[ab]

Means in each column followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

roots revealed extensive colonization by endophytic fungi with a 90% level of infection. Examination showed the presence of eight distinct species of AM fungi, viz. *Glomus aggregatum, G. geosporum, G. macrocarpum, G. mosseae, Acaulospora scrobiculata, Gigaspora margarita, Sclerocystis pakistanica,* and *Scutellospora calospora.*

The growth response and mycorrhizal development in kashini raised in sandy loam red soils were assessed for the impact of inoculation with different indigenous AM fungi and response was found to vary. Inoculated plants generally had greater plant height, shoot, and root biomass, number of leaves, length, and width of leaves (Table 1) as compared to the uninoculated controls. Plants inoculated with *Gigaspora margarita* showed increase in all growth parameters studied followed by *Glomus aggregatum, Glomus mosseae,* and *Glomus macrocarpum.*

Inoculation of kashini plants with AM fungi resulted in increased root volume, dry matter yield, and leaf yield. However there was no positive correlation between plant growth parameters and mycorrhizal colonization. The phosphorus and potassium content of shoots and roots of inoculated plants was significantly greater than that of uninoculated controls. The P and K content was more pronounced in plants inoculated with *Gigaspora margarita*. This is evident from the P content of soil which showed a marked decrease. A similar result was achieved by Gupta and Janardhan, (1991) and Ponder (1984), with the *Glomus aggregatum* and *G. fasciculatum* association with palmarosa and white ash plants, respectively.

AM fungi are known to improve plant growth mainly through increased uptake of P and other nutrients (Jeffries 1987). Mallesha, Padmavathy, and Bagyaraj (1994) reported that species and strains of AM fungi differed to the extent by which they increased nutrient uptake and plant growth. Hence some researchers suggested the need for selecting efficient AM fungi that can be used for inoculating different plants (Bagyaraj, Byra Reddy, and Nalini (1989); Mallesha and Bagyaraj (1994). Abott and Robson (1982) defined efficiency as the ability of the fungus to increase plant growth in a phosphate-deficient soil. This depends on the ability to form extensive and well-distributed hyphae in soil and extensive root colonization, to absorb P from soil.

Higher root colonization allows more host fungal contact and exchange of nutrients and helps in plant growth (Abott and Robson 1982; Bagyaraj, Byra Reddy, and Nalini 1989). In the present study, inoculated plants had a higher shoot and root dry weight, leaf yield, shoot and root P and K content compared to uninoculated plants in spite of having lesser mycorrhizal colonization than control plants. This could be due to low populations of AM fungi. The present study reveals that kashini plants differ in their response to inoculation with the fungus and confirms that AM fungi do have a host preference (Mallesha, Padmavathy, and Bagyaraj 1994). It also reveals that *Gigaspora margarita* is the best fungus for inoculating kashini plants in sandy loam red alfisol soils.

Acknowledgements

The authors are grateful to Dr Akbar Gowsher, Tamil Nadu, India for providing seeds of kashini and to Kalvi and to Kalvi Kavalar Shri K Thulasiah Vandayar, Secretary and Correspondent, AVVM Sri Pushpam College (Autonomous), Poondi, Thanjavur, Tamil Nadu, India for providing facilities and encouragement.

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Distribution of AM fungi in the rhizosphere of different tea clones

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Introduction

The occurrence of arbuscular mycorrhizal fungi associated with different host plants grown in various type of saline and acidic soils is well documented (Gupta, Basak, and Das 2002; Alloush and Clark 2001). Tea is an important plantation crop of very high economic and commercial value and is reported to have mycorrhizal association (Otieno 1995; Rajagopal and Ramarethianan 1997; Kumaran and Santhankrishanan 1995). The effect of mycorrhization on their vegetative growth and nutrient uptake has been reported (Zhi 1995; Liu and Lei 1995). However, studies on AM association with tea plants in acidic soils are scanty. Therefore, to collect some information on the distribution of AM fungi in low pH soils a preliminary survey was made of the rhizosphere of different tea clones grown in the Tarmakant tea plantations in Keonjhar.

Material and methods

The present study was carried out in the tea plantations at Bhuyanprih Tea Estate situated in

Tarmakanta about 48 km from Keonjhar district of Orissa, India. The aim was to study the distribution of AM fungi in the rhizosphere of 5 tea clones, Betzan, Tenali, Nandadevi, TV1, and TV18 of 15–17 year-old plants. Colonization (%) in tea roots was estimated following the slide method (Phillips and Hayman 1970). Spore population in the rhizosphere soil was counted as per the wet sieving and decanting method (Gerdemann and Nicholson 1963). Microphotography was done using a stereozoom microscope (Nikon Optiphot). The AM fungi isolated from soil were identified on the basis of their morphological characteristics (Schenck and Perez 1987). The soil pH was measured by a Mettler Toledo MA 235 pH/Ion Analyzer.

Results

Arbuscular mycorrhizal fungi are found to occur in the rhizospheric soil of different tea clones. The pH of the rhizospheric soil ranged from 4.74 to 5.14 (Table 1). All the tea clones were found mycorrhizal and colonized with vesicles and arbuscules within the range 3%–27% (Table 1). Tenali had the maximum colonization (upto 27%), followed by TV18, Betzan, Nandadevi, and TV1 (Table 1). The spore population (expressed in terms of spore count/100 g soil) varied from 492–940; TV18 had the maximum spore population followed by Nandadevi, TV1, Betzan, and Tenali.

Table 1 Mycorrhizal colonization, spore number and soil pH of tea clones

Tea clones	*pH*	*Colonization %*	*Spore no./100 g soil*
Betzan	4.93–5.05	3–16	593–796
Nandadevi	4.85–4.97	4–12	659–900
Tenali	4.93–5.14	10–27	566–709
TV1	4.74–4.90	6–8	492–880
TV 18	4.83–4.92	10–18	643–940

There were 34 types of AM fungi belonging to the genera *Acaulospora* (4 species), *Glomus* (24 species), *Sclerocystis* (6 species) (Table 2, Plates 1–6). Rhizospheres of both Nandadevi and Betzan clones were represented by all the three genera of AM fungi; the maximum number of species, however, was distributed in the Nandadevi rhizosphere. Tenali was devoid of *Acaulspora*; TV1 and TV18 were lacking *Acaulospora* and *Sclerocystis*. Among four species of *Acaulospora*, three were present in the Nandadevi rhizosphere and one in Betzan. Three species of *Sclerocystis* were recorded in Nandadevi followed by Betzan (two species) and Tenali (one species). *Glomus* species was distributed in all the rhizospheres with maximum frequency in the soil of the Nandadevi rhizosphere.

Table 2 Distribution of AM spores in the rhizospheric soil of different clones of tea

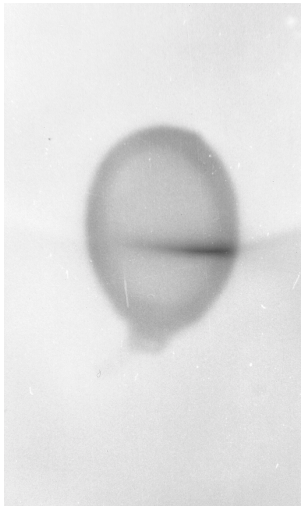
	Tea clone				
AM Fungus	*Betzan*	*Nandadevi*	*Tenali*	*TV–1*	*TV–18*
Acaulospora sp. 1	–	+	–	–	–
Acaulospora sp. 2	–	+	–	–	–
Acaulospora sp. 3	+	–	–	–	–
Acaulospora sp. 4	–	+	–	–	–
Glomus sp. 1	–	–	–	+	–
Glomus sp. 2	–	–	–	+	–
Glomus sp. 3	–	+	–	–	–
Glomus sp. 4	–	+	–	–	–
Glomus sp. 5	–	+	–	–	–
Glomus sp. 6	–	+	–	–	+
Glomus sp. 7	–	+	–	–	+
Glomus sp. 8	–	–	+	–	–
G. albidum	–	–	–	+	–
G. australe	–	+	–	–	–
G. citricola	–	+	–	–	–
G. constrictum	–	+	–	–	–
G. delihence	–	–	+	–	–
G. fasciculatum	+	–	–	–	–
G. flavisporum	–	+	–	–	–
G. fuegianum	–	–	+	–	–
G. glomerulum	–	+	–	–	–
G. magnicaulis	–	+	–	–	–
G. microcarpum	–	+	–	–	+
G. multicaule	–	–	+	–	+
G. pulvinatum	+	–	–	–	+
G. tenebrosum	–	+	–	–	+
G. versiforme	–	+	–	–	–
G. vesiculiferum	–	+	–	–	+
Sclerocystis sp. 1	+	–	–	–	–
Sclerocystis sp. 2	–	+	–	–	–
S. dussiiq	–	–	+	–	–
S. microcarpa	–	+	–	–	–
S. pakistanica	+	–	–	–	–
S. sinuosa	–	+	–	–	–

+ = AM fungi present
– = AM fungi absent

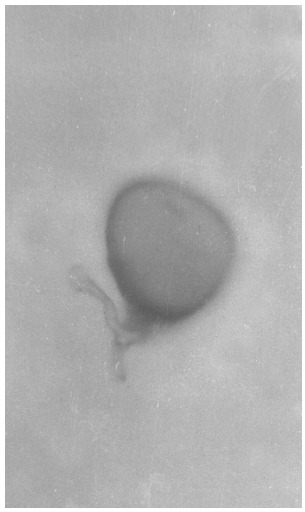
Discussion

The present study was important as few reports document the incidence of AM fungi in acid soils (Rajagopal and Ramarethianan, 1997; Kumaran and Shanthankrishanan 1995). The mycorrhization of tea plants supports the findings of Otieno and Zhi (1995). Though Tenali roots had the maximum colonization, yet AM fungi were widely distributed in Nandadevi. Dominance of *Glomus* species in the soils of tea plantations was evident but *Acaulospora*, *Sclerocystis* were also found. The occurrence of *Gigaspora* was not observed in this acidic soil though its presence was reported earlier in acidic soils of other tea plantations (Kumaran and Shanthankrishanan 1995). This varied occurrence

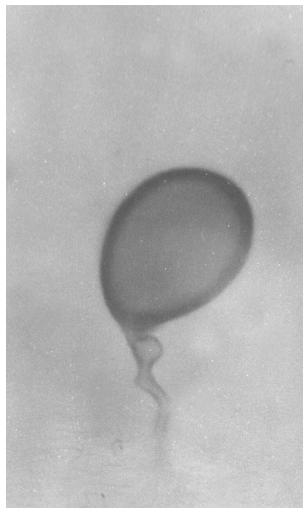
Plate 1



Acaulospora sp. 1



Acaulospora sp. 2



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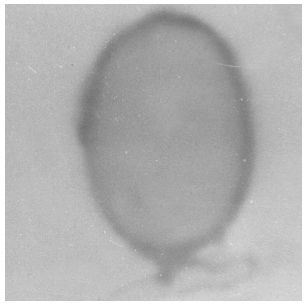
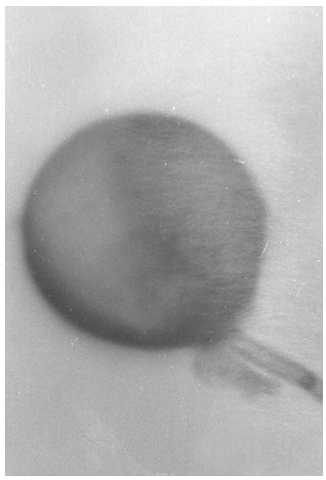
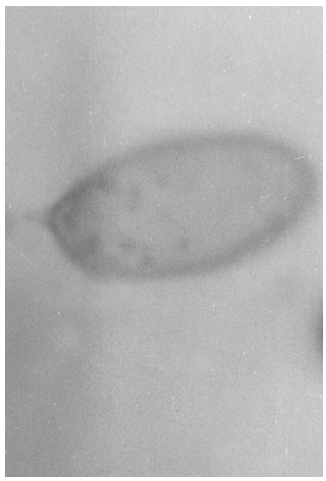


Acaulospora sp. 4

Plate 2



Glomus sp. 1



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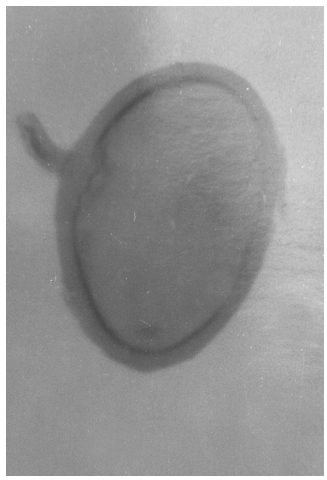
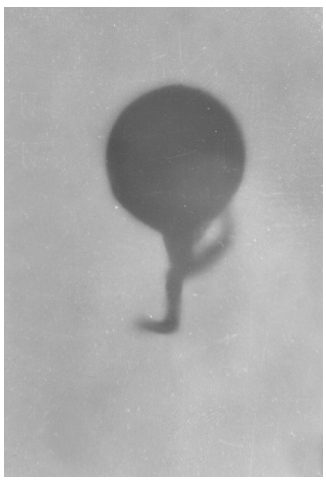
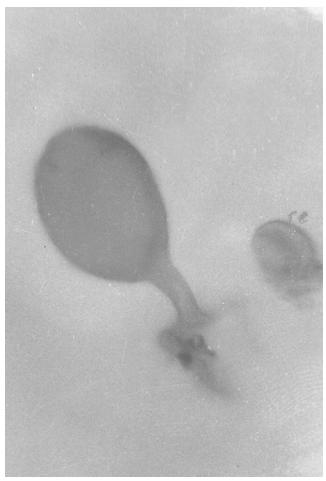


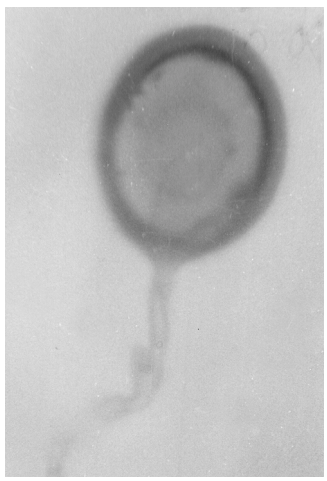
Glomus sp. 3



Glomus sp. 4



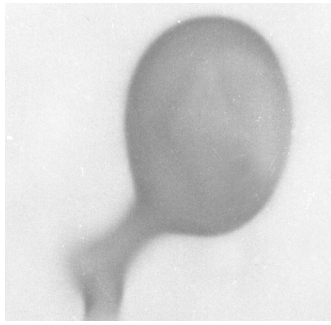
Glomus sp. 5



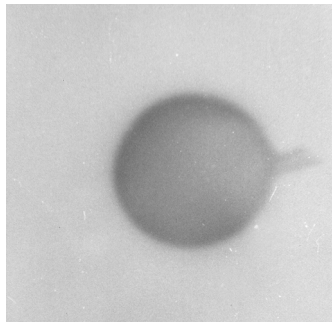
Glomus sp. 6

Plate 3

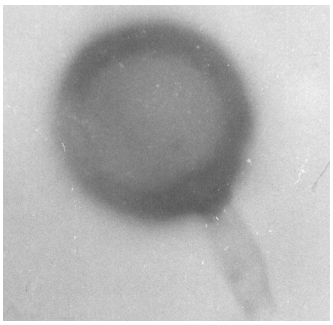
Glomus sp. 7



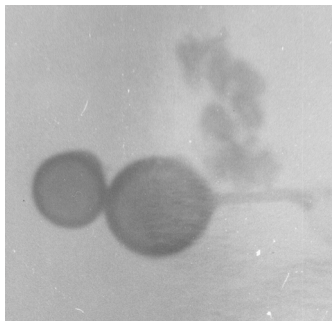
Glomus sp. 2



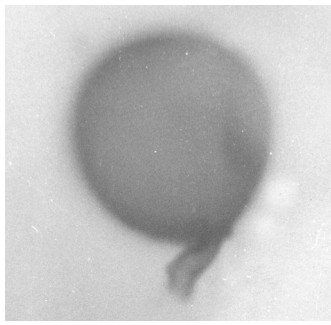
G. albidum



G. australe

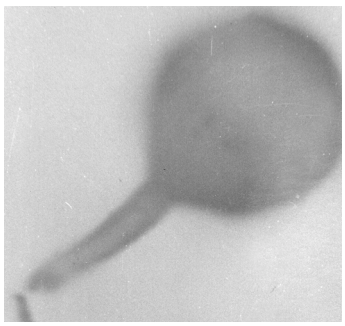


G. citricola

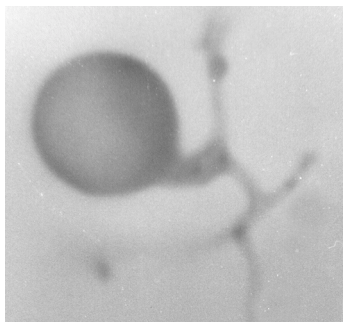


G. constrictum

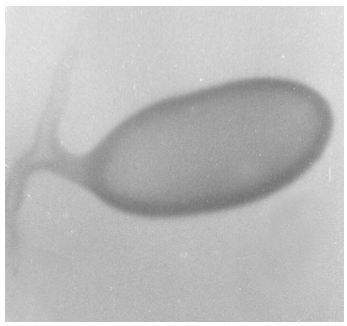
Plate 4



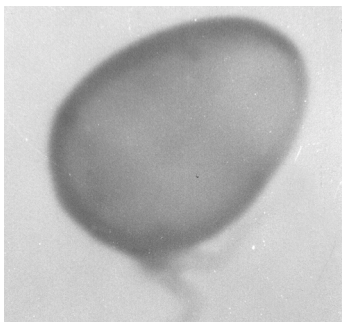
G. delihence



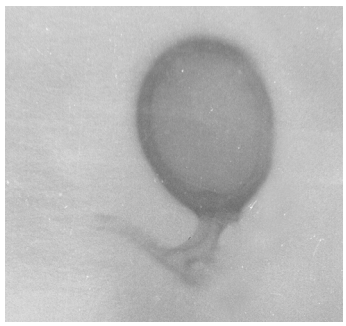
G. fasciculatum



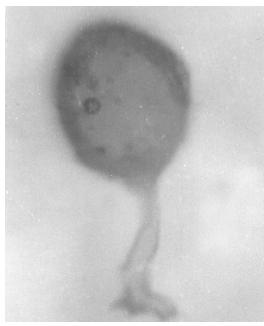
G. flavisporum



G. fuegianum

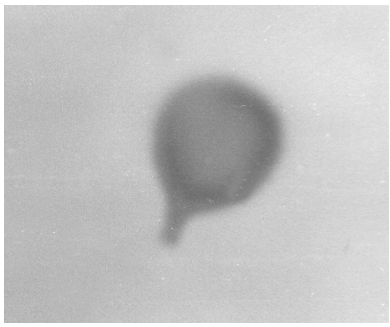


G. glomerulum

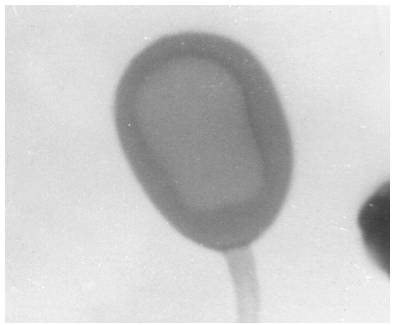


G. magnicaulis

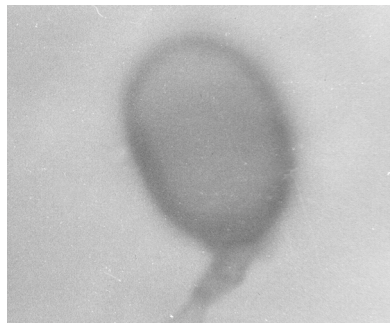
Plate 5



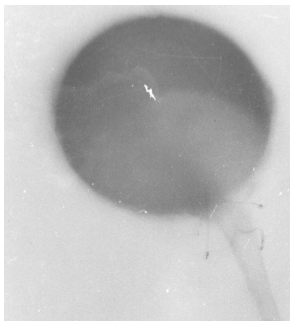
G. microcarpum



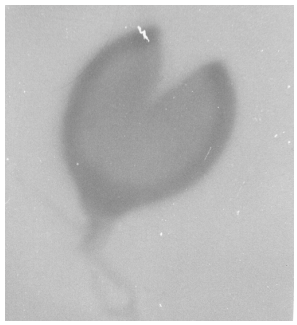
G. multicaule



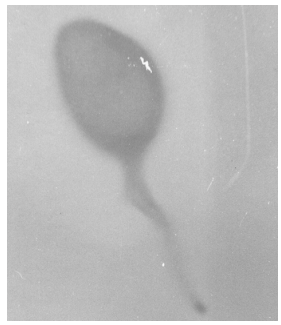
G. pulvinatum



G. tenebrosum

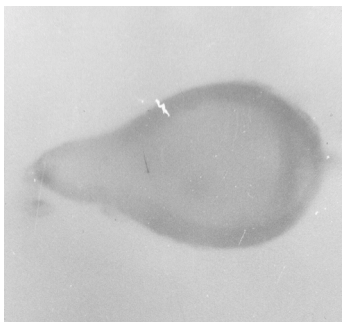


G. versiforme

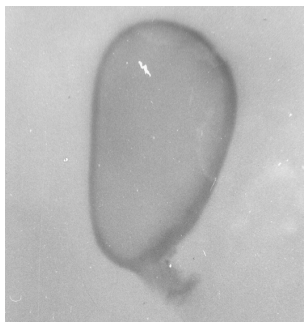


G. vesiculiferum

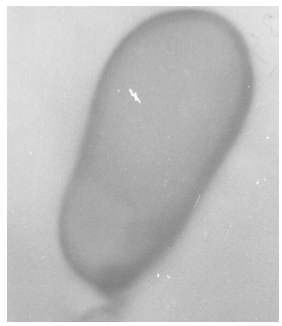
Plate 6



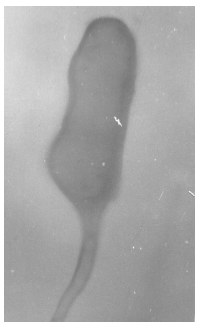
Sclerocystis sp. 1



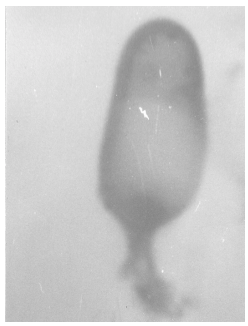
Sclerocystis sp. 2



S. dussiiq



S. microcarpa



S. pakistanica

S. sinuosa

may be due to the host plant specificity, edaphic, and other environmental factors. Factors associated with soil acidity are considered to be limiting for plants (Kidd and Proctor 2001). They suffer from phosphorus deficiency and growth is affected adversely (Gupta and Krishnamurthy 1996). The occurrence of AM fungi in such acidic soils encourages us to exploit them for improving plant productivity. The present maiden survey is also important from the point of view of obtaining new strains of AM fungi of interest.

Acknowledgement

Authors are thankful to M/s Bhuyanpirh Tea Estate, Tarmakant, Keonjhar, Orissa for help during the present study.

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Association of AM fungi with Indian borage (*Plectranthus amboinicus* (Lour.) *Spreng*) and its influence on growth and biomass production

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<*hemaravvy@yahoo.com*>

The introduction of beneficial organisms into soil is one of the present cruxes of applied mycorrhizal research. AM (arbuscular mycorrhizal) fungi are common inhabitants of the roots of several plants and the role of mycorrhizal associations in the mobilization and uptake of phosphorus and productivity of many leguminous and other crops is well documented (Jeffries 1987). There are few published reports on the influence of AM fungi on the growth and nutrition of medicinal plants (Gupta and Janardhanan 1991; Selvaraj 1989). Indian borage (*Plectranthus amboinicus* Lour. Spreng) is an important medicinal plant grown in the herbal gardens of Thanjavur, India. The leaves are used for the treatment of urinary diseases, epilepsy, chronic asthma, cough, bronchitis, and malarial fever and it also acts as a powerful aromatic carminative. Because of increased demand it is cultivated in well fertilized and irrigated soils. Some plants growing in poorly nutritive and unfertilized soils were found thriving luxuriantly and it was suspected that this was at least partly due to a mycorrhizal relationship. However, no information is available about any mycorrhizal association in this medicinal plant

and hence, an investigation of the role of such an association on growth, biomass production, and nutritional uptake was carried out.

Materials and methods

A number of Indian borage plants growing in unfertilized and nutritionally poor soils were collected from the herbal garden on the campus of the college during the months of June – July 1999, with an almost complete root system and the surrounding soil. The roots were carefully washed, cleared with 10% KOH, stained with trypan blue (Phillips and Hayman 1970). The percentage of root colonization was based on the number of root segments colonized by AM fungi. AM fungal propagules were isolated from the rhizospheric soil using the wet sieving and decanting technique (Gerdemann and Nicholson 1963). The number of AM propagules present in soil was calculated per 100 g dry weight of the soil. The species level identification of different AM fungi was done following the keys provided by Trappe (1982) and Schenck and Perez (1990).

Pot cultures were raised by inoculating eight species of predominant AM fungi extracted from soil on onion (*Allium cepa* L.) seedlings growing separately in steam-sterilized soil in pots. After four months, the plants were harvested separately and the roots examined for AM colonization. Root pieces and soil mixture from onion separately with eight test fungi (Table 1) were used as mycorrhizal inocula for greenhouse experiments. Greenhouse experiments assessed the effect of the different AM fungi on growth and biomass production of the host. Seedlings were raised from surface-sterilized seeds in steam-sterilized soil. Experiments were conducted in 20 cm pots filled with sandy loam soil. The pots were sterilized by steam at 121°C for 2 h. The potting mix had a pH of 7.4 and 2.4 µg available P per gram. In this experiment eight different indigenous AM fungi (as in Table 1) maintained as pot cultures on onion were screened against Indian borage (*Plectranthus amboinicus* Lour. Spreng) for symbiotic response. Inoculum at the rate of 10,500 infective propagules per planting point based on the most probable number method (Porter 1979) was added at the time of transplanting.

Table 1 Influence of different native AM fungi on growth response of *Plectranrthus amboinicus (Mean of five replicates)*

	Plant height (cm)		*Plant dry weight* (g/plant)		*AM colonization in roots* (%)	*Number of AM spores/100 g of soil*	*Essential oil content* (%)
Treatment*	*Shoot*	*Root*	*Shoot*	*Root*			
Control (uninoculated)	62.0[a]	23.5[a]	16.5[a]	12.5[a]	0[a]	0[a]	0.55[a]
Acaulospora morrowae	78.6[b]	33.2[b]	20.6[b]	18.4[b]	54.0[a]	420[d]	0.59[b]
Gigaspora margarita	78.5[b]	34.2[b]	23.2[c]	19.6[b]	74.0[b]	560[b]	0.60[b]
Gigaspora aggregatum	90.6[c]	52.4[c]	24.5[c]	23.5[c]	92.0[c]	860[c]	0.61[b]
Glomus ambisporum	84.5[b]	42.0[c]	23.8[c]	19.8[b]	65.0[d]	480	0.58[a]
Glomus deserticola	86.0[c]	46.0[c]	24.0[c]	20.4[b]	68.0[d]	570[b]	0.58[a]
Glomus geosporum	72.4[b]	34.5[b]	20.2[b]	19.8[b]	82.0[b]	620[b]	0.59[b]
Sclerocystis sinuosa	66.4[a]	28.6[a]	19.8[b]	17.5[a]	45.0[a]	380[a]	0.57[a]
Scutellospora calospora	68.5[d]	29.2[a]	19.9[b]	17.6[a]	63.0[d]	460[d]	0.57[a]

*Means (n = 5) in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

Table 2 Influence of different native AM fungi on the shoot and root P and K content of *Plectranthus amboinicus (mean of five replicates)*

	Phosphorus content		*Potassium content*	
Treatment	*Shoot mg/plant*	*Root mg/plant*	*Shoot mg/plant*	*Root mg/plant*
Control (uninoculated)	73.4[g]	10.9[f]	56.2[d]	9.2[f]
Acaulospora morrowae	98.5[e]	26.2[d]	64.0[b]	14.5[d]
Gigaspora margarita	142.0[b]	33.5[c]	65.0[a]	21.4[b]
Gigaspora aggregatum	168.5[a]	45.4[a]	66.2[a]	22.4[a]
Glomus ambisporum	133.2[e]	45.0[a]	63.4[b]	20.5[b]
Glomus deserticola	122.6[d]	40.0[b]	62.2[c]	20.4[b]
Glomus geosporum	131.3[c]	33.6[c]	60.0[c]	19.8[c]
Sclerocystis sinuosa	90.2[f]	24.0[e]	59.4[d]	13.2[e]
Scutellospora calospora	99.8[e]	23.8[c]	60.4[c]	15.4[d]

Means (n = 5) in each column followed by the same letter are not significantly different (P < 0.05) from each other according to DMR test.

One set of plants without inoculation was the control. No fertilizer was applied throughout the growth period and the plants were watered using sterilized tap water. The treatments were arranged following a randomized block design.

Eighty five days after transplanting, plants were harvested for determining the mycorrhizal status and growth response. The plant samples were oven dried at 60°C to a constant weight to determine the plant biomass. The phosphorus and potassium content of the plants was determined after tri-acid digestion (Jackson 1973). The essential oil content of AM associated plants and non-mycorrhizal (control) plants was determined by hydro-distillation using Clevenger's apparatus. The root system was removed for assessing AM infection by the grid-line intersect method (Giovannetti and Mosse 1980), after clearing the roots with 10% KOH and staining with trypan blue (Phillips and Haymann 1970). The data were analyzed following the randomized complete block design and the treatment means were separated by Duncan's multiple range (DMR) test.

Results and discussion

In the field survey, a number of Indian borage plants growing in a nutritionally poor sandy loam soil were found to grow almost as well as the cultivated plants. Microscopic examination of their roots revealed extensive colonization by AM fungi with 92% level of infection. A large number of inter and intra-matrical vesicles were between 120 µm and 140 µm in size. The vesicles were globose to subglobose and the subtending hyphae were simple. Based on the morphological characters, the AM isolate was identified as a species of Glomus. Altogether eight AM fungi were isolated from root zone soils and identified (Table 1). Among them *Glomus aggregatum* and *Glomus ambisporum* were predominant. However, *Gigaspora, Acaulospora, Sclerocystis*, and *Scutellospora* rarely occur. Soilborne auxiliary cells of *Gigaspora* and *Scutellospora* were also isolated.

The growth response and mycorrhizal development of plants raised in sandy loam soils were assessed for the impact of inoculation with different AM fungi. The response of the Indian borage plant to inoculation with different AM fungi varied. Inoculated plants generally had greater plant height, biomass production, and nutrition uptake (Table 1) compared to the uninoculated controls. However, no significant increase in essential oil content was found. Plants inoculated with *Glomus aggregatum* showed increase in all growth and nutritional parameters studied, followed by *Gigaspora margarita, G. deserticola, G. geosporum, G. ambisporum, Scutellospora calospora, Acaulospora marrowae,* and *Sclerocystis sinuosa.* However, *S. sinuosa* did not show any significant difference from the control (Table 1).

Inoculation of *Plectranthus* plants with AM fungi resulted in increased plant height, dry matter, and nutrient content. However, there was no positive correlation between plant growth parameters and mycorrhizal colonization. Mycorrhizal treatments resulted in an increase in the number of spores in the rhizosphere soil and this was maximum in *Glomus aggregatum* followed by *G. geosporum* (Table 1). The phosphorus and potassium content of the shoot and roots of inoculated plants was significantly higher compared to the uninoculated control (Table 2). The P content was more pronounced in plants inoculated with *G. aggregatum* followed by *Gigaspora margarita*. This was also evident from the decrease in the P content of the soil. A similar result was achieved by Ponder (1984) when he studied *Glomus fasciculatum's* association with white ash and black walnut plants and *Glomus aggregatum's* association with *palmarosa* (Gupta and Janardhanan 1991).

Arbuscular mycorrhizal fungi are known to improve plant growth mainly through increased P uptake and other nutrients (Jeffries 1987). Bagyaraj, Byra Reddy, and Nalini (1989) reported that different strains of AM fungi differ to the extent by which they increase nutrient uptake and plant growth. Hence some workers suggested the need for selecting efficient AM fungi that can be used for inoculating different plants (Bagyaraj, Byra Reddy, and Nalini 1989).

In the present study, AM status was considerably higher in all inoculated treatments compared to the control plants. The extent of colonization varied with AM fungi. Higher root colonization allows more host-fungus contact and the exchange of nutrients helps in better growth (Mallesha and Bagyaraj 1995). AM fungi differ greatly in their symbiotic effectiveness which depends on their preference for particular soils or host plant specificity (Dhillion 1992), direct ability to stimulate plant growth, rate of infection, competitive ability, and tolerance to applied chemicals. These results indicate that the natural population of Indian borage is associated with AM fungi, predominantly by species of *Glomus*. This could be the reason for its uninhibited growth even under unfavourable soil nutritional conditions. *Glomus aggregatum* had a wide host range. Although it has been found to be associated with a large number of grasses, this is the first report of this endophyte in Indian borage. In this study, *Glomus aggregatum* caused higher plant growth, biomass production, percent root colonization, number of spores, and uptake of P and K as compared to other AM fungi and it can be considered an efficient AM fungus for *Plectranthus amboinicus* in sandy loam soil.

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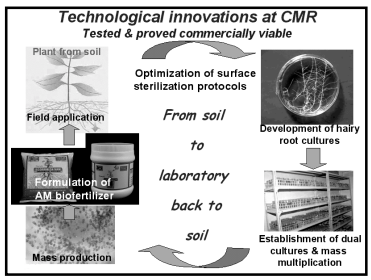
TERI' s mass inoculum production technology of AMF: its commercialization and farmers' field demonstrations

Arbuscular mycorrhiza fungi (AMF) are well established as plant- promoting beneficial fungi. They are known to occur in soil universally, but are found in fewer numbers in tropical conditions. Several methods for mass inoculum production have been designed, but for several reasons they did not gain commercial success. The Centre for Mycorrhizal Research, TERI, India, has deve-loped a technology for mass production of AM fungi. The programme was partially supported by Department of Biotechnology, Ministry of Science and Technology, Government of India. This biofertilizer technology constitutes a supplementary source of nutrients to high input-intensive agriculture. The technology benefits small and marginal farmers who stand in immediate need of the low-cost input to increase the productivity of grains, legumes, horticultural crops, and trees. Incorporation of Vesicular-Arbuscular Mycorrhiza (VAM) is specifically important for cash crops, horticultural, and plantation crops of commercial value, tree species important in agro-forestry, and for wasteland development programmes. Highly mycotropic plants are characteristic of stable, sustainable ecosystems, and any attempt to convert non-sustainable ecosystems into sustainable ones acknowledge the mycorrhizal component.



The root organ culture (ROC) technique exploited for the technology development, enables the production of bulk inocula offering pure, viable and contamination-free healthy propagules, which are otherwise produced in fewer numbers with interfering microbes by other techniques of bulk production. Several rate-limiting factors have been dealt with during the course of technology development. Before being handed over to the industry for commercialization, the technology was extensively tested in diverse agro climatic regions across the country using a wide range of plant species ranging from agricultural, horticultural, floricultural, and ornamental importance along with forestry species.

The finished technology was transferred to two leading industries in India. This was subsequently implemented and the finished mycorrhizal product is now commercially available. The first commercial product was officially launched by Prof M M Joshi, Hon'ble Minister of Science and Technology, on March 2002. Different formulations have been made for convenience of application, ranging from powders for broadcasting in fields to tablets for forestry plantations. Several attempts have been made to cut the cost of the finished product so as to make it accessible and yet commercially viable while maintaining the superiority of the quality of the product.

Several checks have been implemented to ensure formulations at the industrial level. AMF now have the potential to gain tremendous importance in the effort to reduce chemical fertilizer application for sustainable food production particularly among the marginal farming community.

New approaches

Importance of particle size of soilless substrate in VAM inoculum production

The size of particles in a soilless substrate plays an important role in optimizing production of VAM inocula. Atimanav and Alok Adholeya (2000) graded locally available river sand into four texture grades with particle sizes 1.70–0.78 mm (Grade A), 0.78–0.50 mm (Grade B), 0.50–0.25 mm (Grade C) and less than 0.25 mm (Grade D). Each grade of sand was filled in pots, inoculated with 50 ml of freshly produced inoculum of *Glomus intraradices* consisting of soil, extra radical spores, hyphae, and infected sorghum root pieces and planted with maize (*Zea mays*) plants (*Mycorrhiza* **10**: 43–48, 2000). After 12 weeks growth, maize plants in pots with Grade B and Grade C sand supplied with Hoagland nutrient solution with or without phosphorus, had higher root fresh weights, spore production and percent mycorrhizal colonization than in plants growing on sand of other particle sizes.

The production of spores and infectious propagules was enhanced by the nutrient solution without phosphorus. Experiments were also conducted by the authors on the use of clay-brick granules, charcoal, coal marl, sand or perlite, each with optimum particle size between 0.50 and 0.78 mm, as substrate. The percent root length colonized by *Glomus intraradices* and production of infectious propagules was 40%–50% higher in plants growing in clay-brick granules and sand than on other substrates. VA-mycorrhizal colonization ranged from 29% in perlite to 71% in clay-brick granules. For spore production, sand was the best substrate, followed by charcoal, clay-brick granules, coal marl, and perlite. Dry shoot weight was highest in plants gown in clay-brick granules and sand. Root growth was higher in clay-brick granules than in plants grown on other substrates.

Recent references

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

This list consists of papers from the following journals.

- *Chemosphere*
- *Communications in Soil Science and Plant Analysis*
- *Ecological Modelling*
- *Ecological Monographs*
- *Environmental Signal Processing and Adaptation*
- *Journal International Des Sciences de La Vigne et du Vin*
- *Journal of Biotechnology*
- *Journal of Mycology and Plant Pathology*
- *Molecular Ecology*
- *Mycorrhizal Technology in Agriculture: from Genes to Bioproducts*
- *Mycorrhiza*
- *Mycotaxon*
- *Plant and Cell Physiology*
- *Plant Physiology*

Copies of papers published by mycorrhizologists during this quarter may please be sent to the Network for inclusion in the next issue.

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos [*address of the corresponding author]
Bi Y L, Li X L, and Christie P. 2003	**Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus soil amended with zinc and phosphorus** *Chemosphere* **50**(6): 831–837 [* Li X L, China Agricultural University, College of Agriculture Resources and Environmental Science, Department Plant Nutrition, Beijing 100094, Peoples Republic of China]
Chen B D, Li X L, Tao H Q, Christie P, and Wong M H. 2003	The role of arbuscular mycorrhiza in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc *Chemosphere* **50**(6): 839–846 [*Li X L, China Agricultural University, Department of Plant Nutrition, Beijing 100094, Peoples Republic of China]

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos [*address of the corresponding author]
Bi Y L, Li X L, Christie P, Hu Z Q, and Wong M H. 2003	**Growth and nutrient uptake of arbuscular mycorrhizal maize in different depths of soil overlying coal fly ash** *Chemosphere* **50**(6): 863–869 [*Li X L, China Agricultural University, Department of Plant Nutrition, Beijing 100094, Peoples Republic of China]
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Centre for Mycorrhizal Culture Collection

Effect of crop rotation on diversity and functionality of arbuscular mycorrhizal fungi (AMF)

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The search for self-sustaining, low-input, diversified, and energy-efficient agricultural systems is now a major concern for many researchers, farmers, and policy-makers worldwide. A key strategy in sustainable agriculture is to restore the functional biodiversity of the agricultural landscape (Altieri 1995). Biodiversity performs key ecological services and can lead to agroecosystems capable of supporting their own soil fertility, crop protection, and productivity. Different options to diversify cropping systems are available depending on whether the current monoculture systems to be modified are based on annual or perennial crops. Rotation and multiple cropping systems are effective management strategies for annual monocultures.

Cropping activities go on all throughout the year in India, provided water is available. In northern India, there are two distinct seasons, *kharif* (July to October), and *rabi* (October to March). Crops are grown solo or mixed (mixed-cropping), or in a definite sequence (rotational cropping). The land may be sown with one crop during one season (mono cropping), or by two crops (double cropping), which may be grown one after the other in a single year. Of late, the trend has been to sow more than two crops (multiple cropping) in a year. Intensive cropping may be carried out either sequentially or in a relay, when one crop is undersown in a standing crop. Among the *kharif* crops, rice, jowar, bajra, maize, groundnut, and cotton are the predominant base crops and among the *rabi* crops, wheat, barley, oats, jowar, and gram are the main base crops. Generally, wheat and gram are concentrated in the subtropical regions of northern India, while the *rabi* sorghum, is grown mostly in the Deccan. The *kharif* jowar, groundnut, oilseeds, cotton, small millets, and fodder are alternatives to wheat and gram.

Crop mixtures are widely grown. Mixed cropping is generally regarded as the most efficient way of using land. During the *rabi* season, especially in the unirrigated areas of the north, wheat and barley, and wheat and gram or wheat, barley and gram are mixtures of grain crops. *Brassica* and safflower are grown mixed with gram or even with wheat. The yield benefits of crop rotation have long been recognized and exploited.

Since AMF are obligate plant symbionts, it is not surprising that cropping sequences influence their populations. The density of mycorrhizal inocula declines when soils are kept fallow for extensive periods (Black and Tinker 1979; Thompson 1987). Many crops including wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench), sudangrass (*Sorghum sudanense* (Piper) Stapf), sunflower (*Helianthus annuus* L.), soybean (*Glycine max* (L.) Merr.), and corn, grow poorly after long fallows (>12 months). This phenomenon is known as long fallow disorder. Thompson (1987, 1991) provided convincing evidence that long fallow disorder is caused by a decline in viable AM propagules and low levels of mycorrhizal colonization. In replicated field trials with corn and linseed (*Linum usitatissimum* L.), Thompson (1991) showed that densities of AM fungal spores before sowing, percent colonization, and yields were inversely related to the length of time that soil were left fallow. Furthermore, yield response to P and Zn fertilization was positively related to fallow length, which strongly suggests that mycorrhizae are involved in this effect. AM inocula build up during cropping (Thompson 1994). However, not all crops are equal in this regard. Crop species that are non-hosts of AM are equivalent to fallow in their effects on AM populations (Black and Tinker 1979; Ocampo, Martin, and Hayman 1980; Thompson 1991). Host species differ in terms of their residual values of AM inoculum for a subsequent crop. This probably depends not only on the percent colonization of the root system with AM, but also on the total mass of mycorrhizal root produced (Thompson and Wildermuth 1989). Some plant species may have other properties that make them superior sources of AM spores (Struble and Skipper 1985). Selecting the crop species to be grown before a highly mycorrhizal-dependent crop species is therefore an important management factor. By growing hosts that leave a high density of AM propagules in the soil, natural AM populations can be effectively managed to achieve results that might be considered the objective of artificial inoculation.

AMF have been found to associate with plants with relatively low specificity and therefore the

association has been thought to be largely non-specific (Smith and Read 1997). Although there is growing evidence of host-specific differences in plant responses to AMF and fungal response to plants (Bever 2002), not many studies have been carried out to determine the effect of host on the AMF species diversity.

The objective of the present investigation was to study the effect of crop rotation on the development of AMF, including the diversity. Five fields of the wheat cropping system under conventional system (120–150 kg/ha N, 40–60 kg/ha P_2O_5, 40–60 kg/ha K_2O, 25 kg/ha Zn) were selected from Ghaziabad, in the western-plain zone of Uttar Pradesh, India. The selected fields differed in the rotation pattern with wheat. Three different rotations were selected: wheat-rice, wheat-jowar and wheat-sugarcane. In the wheat-rice rotation, three fields were selected:

1 Wheat cultivated alone first followed by rice
2 A crop mixture of wheat and gram cultivated together, followed by rice
3 Wheat cultivated first, followed by *Sesbania* and rice.

In the wheat-jowar and wheat-sugarcane rotation, wheat was grown alone.

In all the selected fields, the specified rotations were in practice for more than five years. All fields shared a common soil order i.e. entisols of alluvial soil. Rhizosphere samples were collected (soil and roots) during the wheat harvesting stage (April 2001). All the soil samples were analyzed for chemical properties (pH, EC, NPK and organic carbon) and mycorrhizal parameters (species diversity, population density, infectivity potential, vital staining of spores, total extramatrical mycelial length, and enzyme activity staining (alkaline and acid phosphatases, succinic dehydrogenase) in these mycelia). Trap cultures were also produced and a greenhouse experiment was set up to test the functional efficacy of AMF isolates (collected from these fields) on wheat. The parameters taken to test functional efficacy of AMF isolates on wheat were: shoot and root dry weight, plant height, NPK, and organic carbon content in shoots. The wheat-jowar rotation resulted in the highest diversity, spore count, root colonization, infectivity of AMF species, extramatrical hyphal biomass, and enzyme activity staining. Moreover, the AMF isolate collected from this rotation also had a significantly high dry weight and nutrient uptake in the experimental wheat plants. Wheat-rice fields showed poor AMF development and less efficient AMF isolates. This suggests that host plants may have a role in determining the beneficial effects of AMF.

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Mycorrhiza News

1. Place of Publication:	New Delhi
2. Periodicity of its Publication:	Quarterly
3. Printer's Name: Whether Citizen of India: Address:	Dr R K Pachauri Yes TERI Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003
4. Publisher's Name: Whether Citizen of India: Address:	Dr R K Pachauri Yes TERI Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003
5. Editor's Name: Whether Citizen of India: Address:	Dr Alok Adholeya Yes TERI Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003
6. Name and address of individuals who own the newsletter and partners or shareholders holding more than one per cent of the total capital:	TERI Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003

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Date: 1 April 2003

ISSN 0970-695X Regd No. 49170/89

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Web site http://www.ispmb2003.com

Beijing, **China**
6–11 July 2003

XVth International Plant Protection Congress
Ms. WEN Liping, Secretariat, 15th IPPC C/O Institute of Plant Protection
Chinese Academy of Agricultural Sciences Beijing 100094 China

Fax 0086 10 62815913 • *Tel.* 0086 10 62815913
E-mail ippc2003@ipmchina.net, cspp@ipmchina.net
Web site http://www.ipmchina.cn.net/ippc/index.htm

Ljubljava, **Slovenia**
June 29–July 3 2003

First FEMS Congress of European Microbiologists
http://www.fems-microbiology.org/fems/meetings.htm

Honolulu, Hawaii, **USA**
26–30 July 2003

American Society of Plant Biologists Annual Meeting
American Society of Plant Biologists**,** 15501 Monona Drive
Rockville, MD 20855-2768 USA

Fax 301 279 2996 • *Tel.* 301 251 0560
E-mail info@aspb.org
Web site http://www.aspb.org/meetings/pb-2003/index.cfm

Canada
10–15 August 2003

The Fourth International Conference on Myccorhizae (ICOM4)
ICOM4 Administration, Bureau des Congrès Universitaires
6600, Côte-des-Neiges road, suite 215 Montreal (Quebec)
H3S 2A9, CANADA

Fax (514) 340 4440 • *Tel.* (514) 340 3215
E-mail icom4@congresbcu.com

Brno, **Czech Republic**
1–3 September 2003

XI International Conference on Plant Embryology, 2003
Plant Reproduction: From Mendel to Molecular Biology
Department of Plant Physiology and Anatomy
Masaryk University in Brno, Faculty of Science
Kotlárská 2, 611 37 Brno
Czech Republic

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Printed and published by Dr R K Pachauri on behalf of the The Energy and Resources Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi – 110 003, and *printed at* Multiplexus (India), 94 B D Estate, Timarpur, Delhi – 110 054.