Vol. 14 No. 1
April 2002

About TERI

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

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 information sharing among the network members and makes the growing literature on mycorrhiza available to researchers. Comprehensive databases on Asian mycorrhizologists and mycorrhizal literature (RIZA) allow information retrieval and supply documents on request.

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

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Part II: fruit trees

Effect of Mycorrhiza on plants raised from cuttings

In studies conducted at the Institute of Plant Nutrition, University of Hohenheim, Stuttgart, Germany, one-year-old apple (cv. 26) cuttings were grown for six months in pot culture, with and without *Glomus macrocarpum* in soil collected from a long-term fertilizer field experiment with different P (phosphorus) availability (20, 210 and 280 mg calcium-extractable P/kg). The indigenous VAM fungus propagule density was reduced by 0.5 M rad X-irradiation. At harvest, non-inoculated and inoculated plants had similar proportions of root length-bearing vesicles. Net dry weight of tree cuttings was significantly increased by inoculation only at 20 mg P/kg (+62%). Increasing P availability from 210–280 mg P/kg led to a four-week depression of shoot elongation rate only in the inoculated plants. Uptake of P was significantly enhanced by inoculation at 20 and 210 mg P/kg (+64% and +12%, respectively). On average, inoculated plants had a significantly higher concentration of zinc in the leaves and roots (+16 and +14%, respectively), and of copper in the stem and roots (+13 and +126%, respectively). A lipid content of 0.9–4.5 percent in root dry matter was attributed to the presence of vesicles corresponding to 1.6–8.2 per cent of total root caloric content. As the control plants were also infected, the beneficial effect of VAM on nutrient uptake and growth of apple cuttings was underestimated at all P levels. VAM-potential at the lowest P level was not fully exploited as onset of infection was most certainly delayed because of a decreased photosynthetic rate due to P deficiency (Gnekow and Marschner 1989).

Effect of Mycorrhiza on micropropagated plants

Effect of ectomycorrhiza on rooting

Studies conducted at the Centro Studi Tecnica Frutticola del CNR, Bologna, Italy, on pear shoot microcuttings placed in jars on MS medium amended with IBA at 0, 0.1 or 0.2 mg/litre, and inoculated with 1 ml of *Hebeloma sinapizans* mycelium slurry, showed that in auxin-free medium, rooting percentage after 28 days was increased three fold by *H. sinapizans* inoculation. With 0.2 mg/litre IBA, rooting was slightly enhanced by inoculation (79% compared with 69% in the control). With IBA at 0.1 mg/litre, the rooting was reduced. Root numbers per plantlet were highest in inoculated media, with or without IBA, but most roots were formed with IBA at 0.2 mg/litre. Root lengths, were however greatest in auxin-free media and were markedly reduced by the addition of the IBA or of *H. sinapizans* inoculum at low concentrations (Baraldi and Branzanti 1988).

Studies conducted at the University of Lyon 1, CNRS, Umr Microbienne Soil, Villeurbanne, France, on rooting of micropropagated cuttings of *Prunus avium* and *P. cerasus*, mainly endomycorrhizal plants, showed that rooting percentage of *P. avium* cuttings was approximately 16% in the absence of hormonal treatment; it increased up to 30% in the presence of 5.7 µM IAA and was enhanced in the absence of IAA but in the

* Compiled from TERI database – Riza

presence of various strains of *Hebeloma cylindrosporum* rooting ranged from 50%–60% with the IAA over-producing mutant D 111 or the wild-type dikaryon D1 to 100% in the presence of the mutants 331 or D 117 of *H. cylindrosporum*. Rooting was approximately 40% in cuttings of *P. cerasus* in the absence of hormonal treatment and except for the mutant D 117, rooting was not significantly improved by *H. cylindrosporum*. In *P. avium*, survival for uninoculated cuttings was 50% and in inoculated cuttings, survival ranged from 30%–100% depending upon the fungal genotype. In *P. cerasus*, survival was 85%–100% and fungi either did not significantly improve survival or lowered it. At acclimatization, fungal hyphae could be observed in close contact with adventitious roots, but did not establish mycorrhizae. Shoot height in *P. avium* was not significantly different in inoculated and uninoculated plants but in *P. cerasus*, growth of acclimatized plants was generally depressed with fungal inoculation (Grange, Bartschi, and Gay 1997).

Effect of VA mycorrhiza on rooting

Studies conducted at the Agricultural Research Centre, Finland, Laukaa, Finland, on micropropagated *Malus* sp. showed that inoculation with AMF did not improve the rooting rate in direct rooting. Also directly rooted microcuttings had a higher survival rate than in vitro rooted cuttings. *Glomus hoi* caused severe rotting of microcuttings and thus lowered the rooting rate of Finnish crabapple cv. Hanna. Sucrose in the in vitro medium gave better support to weaning survival in comparison to glucose and fructose (Uosukainen and Vestberg 1994).

In studies conducted at the Universitat di Torino, Departmento di Biologia Vegetale, Viale, Mattioloi, Turis, Italy, micropropagated plantlets of *Prunus cerasifera* (plum) were inoculated with *Glomus mosseae* or *G. intraradices* or with the ericoid mycorrhizal species, *Hymenoscyphus ericae* and grown in sand culture irrigated for 75 days after transplanting with nutrient solution that included a soluble source of P. None of the treatments significantly affected the length of adventitious roots or the first, second or third order laterals that developed from them. VAM colonization increased the intensity of branching in all root orders with the effect being most obvious on first order laterals where the number of branches increased by 100 to 300 branches per metre. First order laterals made up 55% of the root system in the control and 36% in VAM-inoculated plants but second order laterals made up 25% in the control and 50% in VAM plants. *G. intraradices* and not *G. mosseae* increased root diameter. Both AM fungi increased the concentration of soluble proteins in root extracts and modified the protein profiles by elimination and addition of protein bands detected by PAGE analysis (Berta, Trotta, Fusconi et al. 1995).

In studies conducted at the University of Pisa, Departmento Cultivaz and Difesa Specie Legnose Sez Cultivaz Arboree, Pisa, Italy, micropropagated cuttings of *Prunus cerasifera* clone Mr S2/5 were placed in a sterile rooting mixture or inoculated with AM fungi or uninoculated and given auxin treatment (IBA) of 200 mg per litre or were supplied with a non-sterile sievate of mycorrhizal inoculum. In vivo, rooting of Mr S2/5 shoots was obtained successfully but mycorrhizal inoculum did not show a positive influence on rooting ability of microcuttings. The first harvest of plantlets was carried out very early when mycorrhizal and non-mycorrhizal plants showed no significant growth differences. By this time, AM fungi had greatly increased the proportion of root system present as higher order laterals and also the branching intensity of roots. Also, mycorrhizal inoculation appeared very important for rapid acclimatization and growth of in vivo rooted microcuttings. Mycorrhizal symbiosis induced early resumption of shoot apical growth during the acclimatization phase. No significant alterations in root system morphology were observed in plants grown in unamended soil and also in plants receiving IBA treatments (Fortuna, Morini and Giovannetti 1998).

Studies conducted at the Instituto di Microbiologia Agraria, University of Pisa, Italy, on micropropagated Mr S2/5 plum showed that inoculation with *Glomus mosseae* influenced root morphology since endomycorrhizal plants displayed a more branched root system. Also, inoculation appeared profitable in stimulating shoot apex growth, allowing plants to overcome the prolonged rest period to which micropropagated plantlets of this clone are often subject. However, it was not possible to establish the influence of *G. mosseae* in root induction since even in the uninoculated treatment, rooting percentage reached the maximum (Giovannetti, Fortuna, Loreti et al. 1996).

In studies conducted at the Centro Miglioramento Genetico e Biologia Vite, CNR, Via Leonardo da Vinci, Grugliasco, micropropagated shoots of apple (*Malus pumila* MM 106) were rooted in media containing a vital staining in order to distinguish in vitro-formed roots from in vivo-formed roots. The presence of the dye reduced plant growth in apple. At transfer into the soil, plants were inoculated with the arbuscular mycorrhizal fungus, *Glomus mosseae*. The dye did not prevent root colonization by the mycorrhizal fungus and 4-5 weeks after transplanting, both in-vitro-and in vivo-formed roots were colonized by the VAM fungus (Gribaudo, Zanetti, Morte et al. 1996).

Stimulation of growth by mycorrhizal fungi

Studies conducted at the Centre de Studio per la Microbiologia del Suolo, Via del Borghetto, Pisa, Italy, on apple, peach and plum micropropagated

root stocks, inoculated with *Glomus* sp. (strain A6), on transplanting from in vitro to in vivo culture showed that the optimal root length for effective infection, assessed in apple root stock M25 was 0.1–1.5 cm corresponding to the beginning of root elongation. When inoculated at this stage, plants showed maximum growth increase and survival. Mycorrhizal infection of the Mr S2/5 *Prunus cerasifera* (plum) root stock induced earlier growth renewal after transplanting than in the controls (Sbrana, Giovannetti and Vitagliano 1994).

Studies conducted at the Instituto di Ecofisiologia delle Plante Arboree de Frutto, Bologna, Italy, on micropropagated plantlets of *Pyrus communis* clonal rootstock OHX F51 and *Prunus persica* x *P. amygdalus* clonal rootstock GF 677, inoculated with *Glomus* sp. during the early weaning stage of acclimatization showed that both root stocks were well-colonized though infection of OHX F51 spread more slowly. At the end of initial vegetative growth, mycorrhizal plants of both rootstocks showed a three fold increase in shoot length, longer internodes and greater fresh mass than the controls. The root: shoot ratio was especially altered by VAM inoculation in OHX F51 which showed a greater increase in shoot than root biomass. *Glomus* sp. induced a greater development of both root stocks in the second growing year after over-wintering. Mycorrhizal plants always had a higher content of total soluble sugar although there was no difference in soluble carbohydrate concentration between inoculated and uninoculated plants. Starch accumulation was found only in mycorrhizal plants of the peach x almond rootstock (Rapparini, Baraldi, Bertazza et al. 1994).

Studies conducted at the University of Pisa, Departmento Coltivaz and Difesa Specie Legnose, Sez Coltivaz Arboree, Borghetto, Pisa, Italy, showed that unfertilized and non-mycorrhizal micropropagated plantlets of MM 106 apple (*Malus pumila* L.) and Mr S2/5 plum (*Prunus cerasifera*) showed no apical growth during the post-in vitro acclimatization stage, whereas P fertilization induced early resumption of shoot apical growth. Growth enhancement and percentage of actively-growing apices of plantlets inoculated with *Glomus mosseae*, *G. intraradices* and *G. viscosum* were comparable to those obtained in plantlets fertilized with P. Also, tissue P concentrations of mycorrhizal plantlets were similar to P fertilized plantlets (Fortuna, Citernesi, Morini et al. 1996).

In studies conducted at the Universitat de Torno, Departmento di Biologia Vegetale, Viale Mattioloi, Turin, Italy, micropropagated transplants of *Prunus cerasifera* (plum) were inoculated with either *Glomus mosseae* or *G. intraradices* or with the ericoid mycorrhizal species, *Hymenoscyphus ericae*, and grown in sand culture irrigated for 75 days after transplanting with a nutrient solution that included a soluble source of P. VAM inoculation increased survival and growth by more than 100%, increased root, stem and leaf weight, leaf area, root length and specific leaf area as compared to uninoculated controls or transplants inoculated with *H. ericae*. VAM inoculation decreased the root length/leaf area ratio, root/shoot weight ratio and specific root length. VAM inoculation also increased the uptake of P and its concentration in leaves. The time taken for development of infection varied, being most rapid with *G. mosseae* but was ultimately higher with *G. intraradices* (Berta, Trotta, Fusconi et al. 1995).

Studies conducted at the IRTA, Patovegetal Ctr. Cabrils, Spain, on micropropagated plantlets of almond x peach clone (GF67), grown on two peat-based potting mixtures and tested with four fungi, *Glomus mosseae*, *G. intraradices*, *G.* species (E3) and *Acaulospora laevis* showed that response of VAM fungi varied with the potting mix used (Estaun, Calvet, and Camprubi 1994).

Studies conducted at the Agriculture Research Centre, Finland, Laukaa, Finland, on micropropagated Malus showed that AMF inoculation increased the mean shoot height of established plants, particularly inoculation with *Glomus hoi* V98, *G. claroideum* V439, *G. fistulosum* V128. Some AMF strains like *G. hoi* V 104 caused strong growth retardation. After the rooting and weaning stage, many uninoculated plants lapsed into an arrest of growth and this phenomenon was less frequent in AM inoculated plants (Uosukainen and Vestberg 1994).

In studies, conducted at the CSIC, Estacion Experimental Zaidin, Granada, Spain, mycorrhizal inoculation on micropropagated *Annona cherimola* plants was assayed at two different stages of the micropropagation process. These stages were (i) immediately after the in vitro phase before starting the acclimatization period and (ii) after the acclimatization phase before starting the post-acclimatization period under greenhouse conditions. Plantlet survival was about 50% after the acclimatization period. Plant growth and establishment profited remarkably from mycorrhiza establishment. Most of the VAM fungi assayed greatly increased shoot and root biomass and leaf area. Micropropagated anona plants seemed to be more dependent on mycorrhiza than plants derived from seeds. The greatest effect of VAM on plant growth occurred when they were introduced after the acclimatization period (Azconaguilar, Encina, Azcon et al. 1994).

Studies conducted at the University of Lyon I, CNRS, Umr Microbienne Soil, Villeurbanne, France, showed that shoot height in *Prunus avium* was not significantly different in plants inoculated with *Hebeloma cylindrosporum* and in uninoculated plantlets. In *P. cerasus*, however, growth of acclimatized plantlets was generally depressed with fungal inoculation (Grange, Bartschi, and Gay 1997).

Part III: ornamentals and other plants

Cornus and Cotoneaster

Studies conducted at the Land Resource Research Centre, Agriculture, Canada, on softwood cuttings of Cotoneaster and Cornus, stuck into rooting media containing chopped leek root inocula of either *Glomus intraradices*, *G. clarum*, *G. vesiculiferum* or no VAM, showed that root development was delayed only in *G. intraradices* treatments. Within each plant species, all treatments had similar root weights at the end of the rooting period. Rapid spread of infection resulted in declines in relative root growth and was delayed in Cornus and *G. clarum* treatments. VAM colonization prolonged positive relative root growth after non-mycorrhizal treatments had declined to zero. Cold storage resulted in a significant increase in root infection by *G. clarum* (Nelson and Clough 1987).

Epacris impressa

Studies conducted at the Victorian College of Agriculture and Horticulture, Burnley Campus, Burnley Gardens, Swan Street, Richmond, Victoria showed that *E. impressa* stem cuttings (about 8 cm long) planted in unsterilized soil were mycorrhizal and had a higher survival rate than those planted in sterilized soil which were non-mycorrhizal. The surviving cuttings in the unsterilized soil also had more flowers and roots than those in the sterilized soil. The soil was collected from under the original plants (McLean, Lawrie and Blaze 1994).

Euphorbia pulcherrima

Studies conducted at the USDA, Southeastern Forest Experiment Station, Athens, Georgia, USA showed that the roots of *Euphorbia pulcherrima* cuttings became mycorrhizal when inoculated with *Gigaspora margarita*. Maximum VAM formation occurred following application of azygospores to both the rooting medium in the mist bed and, subsequently, to rooted cuttings at transplant. But in both cases only fewer mycorrhizae developed. *G. margarita* in conjunction with rootone stimulated rooting of cuttings in the mist bed by markedly increasing the number and weight of roots over cuttings treated with rootone alone. The mycorrhizal cuttings withstood transplant shock under high temperatures and low moisture conditions better than non-mycorrhizal cuttings (Barrows and Roncadori 1977).

Gerbera, Nephrolepis and Syngonium

In studies conducted at the University of Laval, Fac Sci Agr and Alimentat, Ctr Res Hort, Quebec City, Canada, micropropagated plantlets of *Gerbera jamesonii*, *Nephrolepis exaltata* and *Syngonium podophyllum* were inoculated with *Glomus intraradices* and *G. vesiculiferum* and potted in peat-based media. Symbiosis was established between the three ornamental species and VAM fungi within 4–8 weeks of culture in the greenhouse, but not during acclimatization. Mortality of *Gerbera* and *Nephrolepis* mycorrhizal plantlets was reduced at week 8 compared to the non-inoculated control. A peat-based substrate low in P and with good aeration improved the spread and efficiency of VAM fungi. The mycorrhizal substrate was beneficial in the long term by increasing leaf and root dry weight of *Gerbera* and *Nephrolepis*. Mycorrhizal *Gerbera* plants flowered significantly faster than non-mycorrhizal plants (Wang, Parent, Gosselin et al. 1993).

In studies conducted at the Centre de Recherche en Biologie Forestiere, Universite Laval, Quebec, Canada, rooted plantlets of in vitro micropropagated *Nephrolepis exaltata* var. *whitmanii*, were transferred to pots containing a brown peat-based mix and simultaneously inoculated with one of the four *Glomus* spp. *Glomus intraradices* and *G. clarum* formed rapid and extensive infection in *N. exaltata* roots, while *G. vesiculiferum* and *G. versiforme* showed a significantly slower rate of infection. Inoculated plants and one control treatment were grown in low phosphorus nutrient solution while in the other control, plants were grown in high phosphorus supplements. The high phosphorus control performed better than all other treatments, except for number of fronds, which did not differ significantly. Out of four mycorrhizal treatments, plants inoculated with *G. vesiculiferum* showed the most significant increase in growth compared with the low phosphorus control (Ponton, Piche, Parent et al. 1990)

Helianthemum almeriense

In studies conducted at the Department de Biologia Vegetal, Universidad de Murcia, Spain, the mycorrhization rate of *H. almeriense* micropropagated plantlets with *Terfezia claveryi*, grown on modified Melin Norkrans agar medium with pH 8.0, was about 80% after 12 weeks. *T. claveryi* did not enhance in vitro rooting of microcuttings of *H. almeriense* but appeared to improve the survival rate of rooted plantlets (Morte, Cano, Honrubia et al. 1994).

Further studies conducted at the above university showed that micropropagated plantlets of *Helianthemum almeriense* formed mycorrhiza in vitro with *Terfezia claveryi* on the recently-developed agar medium MH at pH 7.0. The mycorrhization rate was approximately 75% after 8 weeks. Mycorrhizal association stimulated the growth of the plantlets. *T. claveryi* formed ectendomycorrhizas with a discontinuous mantle of lax hyphae (Morte and Honrubia 1995).

Hydrangea

In studies conducted at the School of Life Sciences, Jawahar Lal Nehru University, New Delhi, India, micropropagated plantlets of hortensia

(*Hydrangea macrophylla*) cv. *leuchtfeuer*) were inoculated with *Glomus intraradices*. At the acclimatization stage, roots were heavily colonized by the VAM fungus. There was 100% survival. Shoot apices were active and no apparent transient transfer shock was noticed. Infected plants had better growth and larger leaf areas than controls. Eighteen-week-old, infected potted plants exhibited considerable resistance to induced-stress environmental conditions in the phytotron. In contrast, non-infected plants were severely wilted and the number of apical buds was reduced. The general bacterial population was several fold higher in the rhizospheres of colonized roots than in non-infected roots with *Pseudomonas fluorescens* being predominant (Varma and Sohuepp 1994).

Morus

In studies conducted at the Mulberry Agronomy Laboratory, Central Sericultural Research and Training Institute, Srirampura, Mysore, India, eight-month, 17 cm long cuttings of mulberry (*Morus* sp) variety S-54, were planted in furrows 15 cm deep and 20 cm apart and inoculated with *Glomus mosseae* inoculum containing spores (more than 15 spores/g of air dried soil) and infected root fragments. The nursery soil was sterilized by burning accumulated weeds for two hours before digging the furrows. Cuttings were planted at 10 cm spacing. An equal number of cuttings was planted on sterilized soil without VAM inoculation as controls. The survival rate of cuttings was 85.9% with inoculation as compared to 76.1% in the controls. Sapling height, average number of leaves per sapling, fresh and dry weight of the plants were significantly increased in inoculated plants as compared to controls. Nitrogen and phosphorus contents of leaves in inoculated plants was more than in the controls (Das, Katiyar, Hanumantha et al. 1994).

In further studies conducted at the above institute on cuttings of three varieties of mulberry (Kanva 2, S-54, S-34) inoculated or not with *Glomus mosseae*, *G. fasciculatum* and mixture of *G. mosseae* + *G. fasciculatum* by the method as described above, significantly higher rates of survival, plant height, plant fresh and dry weight were observed due to VAM inoculation over uninoculated controls. Among inoculations, *G. mosseae* was better followed by mixed inoculation. The variety S-54 responded significantly better than the other varieties. The effect of rock phosphate with VAM inoculation was significantly higher on sprouting, survival, height and fresh and dry weights of plants when compared with other phosphorus sources such as diammonium phosphate and single super phosphate (Fathima, Katiyar, Das et al. 1995).

Pelargonium hortorum

In studies conducted at the Ohio State University, Columbus, Ohio, USA, rooted cuttings of florists' geranium (*Pelargonium hortorum*) were inoculated with spores of *Glomus mosseae*, *G. etunictum* or a suspension lacking mycorrhizal fungi (controls). A complete fertilizer (NPK) or fertilizer lacking phosphorus (NK) were applied at every watering or biweekly for 12 weeks. The highest level of mycorrhizal infection was in inoculated treatments without P. The geraniums were then out planted at 12 field sites with soil P levels ranging from 8 to 230 µg/g. Assessment after 14 weeks showed that inoculated plants receiving NK fertilizer and those receiving NPK fertilizer had significantly greater dry weights, more blooms, and higher performance ratings than comparable non-mycorrhizal plants. There was no difference between inoculated and non-inoculated plants given NPK at every watering. At harvest, plants in all treatments at all sites had become mycorrhizal with no significant differences between treatments in percent mycorrhizal infection, which was 20%–25% of total root length (Chatfield, Rhodes and Powell 1979).

Saccharum officinarum (sugarcane)

In studies conducted at the Department of Botany, Gulbarga University, Gulbarga, India, shoot formation was obtained from leaf explants of sugarcane (*Saccharum officinarum*) variety CO 419 when cultured on MS medium supplemented with 3 mg/litre 2-4 D and 10% coconut milk to obtain a friable sugarcane callus, and further subcultured on MS medium supplemented with 2 mg/litre kinetin and 10% coconut milk for shoot induction. The regenerated shoots were transferred to MS liquid medium using the following treatment for root induction.

1 MS medium supplemented with NAA (5 mg/litre) with 10% coconut milk (T^1)
2 MS medium supplemented with *Glomus aggregatum* spore extract (2500 spores/litre) and 10% coconut milk (T^2)
3 MS medium supplemented with VAM root extract (10 g/litre) and 10% coconut milk (T^3)
4 MS medium with non-VAM root extract (10 g/litre) and 10% coconut milk (T^4)

Root initiation took place after 8–12 days in T^1, 3–6 days in T^2, 2–6 days in T^3 and no rooting took place in T^4. The number of roots per shoot was 10–15 in T^1, 3–12 in T^2 and 30–50 in T^3. The length of roots after 20 days was 0.4–0.6 cm in T^2, 1.5–3 cm in T^2 and 0.5–1cm in T^3. Root length after 40 days was 1–2 cm in T^1, 3–4 cm in T^2 and 1.5–3 cm in T^3 (Muniyamma, Bharti and Reddy, 2000).

Sciadopitys verticillata

Studies conducted at the US Department of Agriculture, Agricultural Research Service, Eastern Regional Research Centre, East Mermaid Lane, Philadelphia, USA, on *Sciadopitys verticilata* showed that a VAM fungus in a peat-based medium significantly increased survival, callus development and rooting percentage of *S. verticilata*

cuttings over non-inoculated cuttings. The presence of a nurse host plant in the absence of *S. verticilata* roots decreased survival and rooting percentage but not callus development, compared to the fungus without the nurse host plant (Douds, Becard, Pfeffer et al. 1995).

Syngonium podophyllum and Draceana

In studies conducted at the Tata Energy Research Institute, New Delhi, India, inoculation of micropropagated plantlets of *Syngonium podophyllum* and *Draceana* sp. by a consortium of VAM fungi during an early-weaning stage of acclimatization showed that *Draceana* exhibited significantly higher colonization than *S. podophyllum* after 20 weeks. Draceana plants showed little difference in the extent of VAM colonization at the weaning or hardening stage but *S. podophyllum* plants were better colonized at the weaning stage than at 20 weeks. In both hosts, survival was high in VAM plants at lower fertility. Inoculated *S. podophyllum* showed better stolon production than in the uninoculated controls at both fertility levels tested though the increase was higher at lower fertility. Draceana showed no response in shoot height. VAM inoculation in both species saved almost 15 days in the total hardening process (Gaur and Adholeya 1999).

Tetraclinis articulata

In studies conducted at the Departmento de Biologia Vegetal, Botanica, Universidad de Murcia, Campus de Espinardo, Murcia, Spain, micropropagated *Tetraclinis articulata* plantlets were inoculated at acclimatization stage with two types of *Glomus fasciculatum* inoculum on sterilized soil, sand and peat mixture. No major differences were observed between the two types of inoculum with respect to the extent of plant root colonization and plant survival. In both cases, mycorrhizal colonization was 37% and it stimulated plant growth and improved the survival of plants during the weaning stage (Morte, Diaz and Honrubia 1996).

Sesbania sesban

In studies conducted at the Jamia Millia Islamia, Centre for Biosciences, New Delhi, India, plantlets of *Sesbania sesban* were developed from somatic embryos and/or adventitious buds (induced from various explants on Gamborg's medium supplemented with 6-benzylaminopurine) in the presence of 10^{-7}M alpha-naphthalene acetic acid and $5x10^{-6}$M gibberellic acid. Subsequent to nodulating the roots with rhizobium, the plantlets were transplanted into sterile garden soil and inoculated with or without *Glomus fasciculatum*. Only 30 per cent of plantlets transferred to soil without *G. fasciculatum* survived. Histochemical studies revealed the presence of intracellular hyphae with well-developed arbuscules and intercellular hyphae with vesicles. This suggested that *G. fasciculatum* formed a good mycorrhizal association with *S. sesban*s roots. The studies indicated that mycorrhizal association with *S. sesbans* roots helped the micropropagated plantlets to successfully withstand transplantation shock (Subhan, Sharmila and Saradhi 1998).

Tobacco

In studies conducted at the Jawaharlal Nehru University, School of Life Science, New Delhi, India, regenerated plantlets of tobacco subjected to two different biological hardening techniques showed 88-94% survival when inoculated with *Piriformospora indica*, a novel plant growth promoting root endophyte, as compared to 62% survival in uninoculated control plants under similar conditions. The tendency of the plantlet to overcome the stress in terms of revival capacity was maximal in the case of *P. indica* as compared to the control (Sahay and Varma 1999).

Viburnum

Studies conducted at the University of Florida, Fort Lauderdale, Florida, USA, showed that inoculation of cuttings of *Viburnum dentatum* during propagation with isolated spores of *Glomus fasciculatum* at the rate of five spores per cubic centimetre of propagating material resulted in uniform infection and increased root growth and root development (Verkade 1987).

References

Azconaguilar C, Encina C L, Azcon R and Barea J M.1994
Effect of arbuscular mycorrhiza on the growth and development of micropropagated *Annona cherimola* plants
Agricultural Science in Finland **3**(3): 281–287

Baraldi R and Branzanti B. 1988
Effect of an ectomycorrhizal fungus on fruit tree micropropagation
Advances in Horticultural Science **2**(2): 75

Barrows J B and Roncadori R W. 1977
Endomycorrhizal synthesis by *Gigaspora margarita* in poinsettia
Mycologia **69**(6): 1173–1184

Berta G, Trotta A, Fusconi A, Hooker J E, Munro M, Atkinson D, Giovannetti M, Morini S, Fortuna P and Tisserant B. 1995
Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera*
Tree Physiology **15**(5): 281–283

Chatfield J A, Rhodes L H and Powell C C. 1979.
Relationship of greenhouse fertilization and mycorrhizal inoculation to outplant performance of florists' geranium
In *Proceedings of the fourth North American Conference on Mycorrhiza* edited by C P P Reid
Colorado: Colorado State University, 197 pp.
[24–28 June 1979, Colorado, USA]

Das P K, Katiyar R S, Hanumantha Gowda M, Choudhury P C, and Datta R K. 1994
An effective technique of VAM inoculation for mulberry (Morus Sp.)
Mycorrhiza News **6**(2): 6–8

Douds D D Jr, Becard G, Pfeffer P E, Doner L W, Dymant T J and Kayser W M. 1995
Effect of vesicular-arbuscular mycorrhizal fungi on rooting of *Sciadopitys verticillata* sieb Zucc. cuttings
HortScience **30**(1): 133–134

Estaun V, Calvet C and Camprubi A. 1994
Arbuscular mycorrhizae and growth enhancement of micropropagated prunus rootstock in different soilless potting mixes
Agricultural Science in Finland **3**(3): 263–267

Fortuna P, Citernesi A S, Morini S, Vitagliano C, and Giovannetti M. 1996
Influence of arbuscular mycorrhizae and phosphate fertilization on shoot apical growth of micropropagated apple and plum rootstocks
Tree Physiology **16**(9): 757–763

Fortuna P, Morini S, and Giovannetti M. 1998
Effects of arbuscular mycorrhizal fungi on in vivo root initiation and development of micropropagated plum shoots
Journal of Horticultural Science Biotechnology **73**(1): 19–28

Fathima P S, Katiyar R S, Das P K, Gowda M Hanumantha, and Choudhury P C. 1995
Influence of vesicular-arbuscular mycorrhizal inoculation on mulberry (Morus sp.) under different sources and levels of phosphorus
In *Mycorrhizae: Biofertilizers for the Future*, pp. 369–373, edited by A Adholeya and S Singh
New Delhi: Tata Energy Research Institute. 548 pp.
[Proceedings of the Third National Conference on Mycorrhiza, New Delhi, India, 13–15 March 1995]

Gaur A and Adholeya A. 1999
Mycorrhizal effects on the acclimatization, survival, growth and chlorophyll of micropropagated *Syngonium* and *Draceana* inoculated at weaning and hardening stages.
Mycorrhiza **9**(4): 215–219

Giovannetti M, Fortuna P, Loreti F, and Morini S. 1996
Effect of arbuscular mycorrhizal inoculation on in vivo root induction of development in shoots of Mr S2/5 plum rootstock grown in in vitro
In *Proceedings of the 3rd International Symposium on Peaches*, edited C G Fidghelli, pp. 99–102
Beijing, China: International Society for Horticultural Science, 374 pp.

Gnekow M A and Marschner H. 1989
Role of VA-mycorrhiza in growth and mineral nutrition of apple (*Malus pumila* var. domestica) rootstock cuttings
Plant and Soil **119**(2): 285–293

Grange O, Bartschi H, and Gay G. 1997
Effect of the ectomycorrhizal fungus *Hebeloma cylindrosporum* on in vitro rooting of micropropagated cuttings of arbuscular mycorrhiza-forming *Prunus avium* and *Prunus cerasus*
Trees - Structure and Function **12**(1): 49–56

Gribaudo I, Zanetti R, Morte M A, Previati A, and Schubert A. 1996
Development of mycorrhizal infection in vitro and in vivo-formed roots of woody fruit plants
Agronomie: Plant Genetics and Breeding **16**: 621–624

Morte M A, Cano A, Honrubia M, and Torres P. 1994
In vitro mycorrhization of micropropagated *Helianthemum almeriense* plantlets with *Terfezia claveryi* (desert truffle)
Agricultural Science in Finland **3**(3): 309–314

Morte M A, Diaz G, and Honrubia M. 1996
Effect of arbuscular mycorrhizal inoculation on micropropagated *Tetraclinis articulata* growth and survival
Agronomie: Plant Genetics and Breeding **16**: 633–637

Morte M A and Honrubia M. 1995
Improvement of mycorrhizal synthesis between micropropagated *Helianthemum almeriense* plantlets with *Terfezia claveryi* (desert truffle)
In *Proceedings of the Fourteenth International Congress on the Science and Cultivation of Edible Fungi*, p. 863–868
[17–22 September 1995, Oxford, UK]

McLean C, Lawrie A C, and Blaze K L. 1994.
The effect of soil microflora on the survival of cuttings of *Epacris impressa*
Plant and Soil **166**(2): 295–297

Muniyamma M, Bharti B K and Reddy C N. 2000.
Effect of VAM on root induction in in vitro sugar cane (*Saccharum officinarum* L.) seedlings—a new technique
Mycorrhiza News **12**(1): 13–16

Nelson S D and Clough K S. 1987
Rooting, growth and infection of two woody ornamentals by three Glomus species
In *Proceedings of the Seventh North American Conference on Mycorrhiza*, edited by D M Sylvia, L L Hung, and J H Graham
Florida: University of Florida, 364 pp.
[3–8 May 1987, Gainesville, Florida]

Ponton F, Piche Y, Parent S, and Caron M. 1990
Use of vesicular-arbuscular mycorrhizae in Boston fern production. II. Evaluation of four inocula
HortScience **25**(4): 416–419

Rapparini F, Baraldi R, Bertazza G, Branzanti B, and Predieri S. 1994
Vesicular-arbuscular mycorrhizal inoculation of micropropagated fruit trees
Journal of Horticultural Science **69**(6): 1101–1109

Sahay N S and Varma A. 1999
***Piriformospora indica*: a new biological hardening tool for micropropagated plants**
FEMS Microbiology Letters **181**(2)

Sbrana C, Giovannetti M and Vitagliano C. 1994
The effect of mycorrhizal infection on survival and growth renewal of micropropagated fruit rootstocks
Mycorrhiza **5**(2): 153–156

Subhan S, Sharmila P, and Saradhi P P. 1998
Glomus fasciculatum alleviates transplantation shock of micropropagated *Sesbania sesban*
Plant Cell Reports **17**(4): 268–272

Uosukainen M and Vestberg M. 1994
Effect of inoculation with arbuscular mycorrhizas on rooting, weaning and subsequent growth of micropropagated malus (L.) Moench
Agricultural Science in Finland **3**(3): 269–279

Varma A and Schuepp H. 1994
Positive influence of arbuscular mycorrhizal fungus on in vitro raised hortensia plantlets
Angewandte Botanik **68**(3/4): 108–115

Verkade S D
Effect of *Glomus fasciculatum* on rooting of *Viburnum dentatum* during propagation
In *Proceedings of the Seventh North American Conference on Mycorrhiza*, edited by D M Sylvia, L L Hung, and J H Graham
Florida: University of Florida. 364 pp.
[3–8 May 1987, Gainesville, Florida]

Wang H, Parent S, Gosselin A, and Desjardins Y. 1993
Vesicular-arbuscular mycorrhizal peat-based substrates enhance symbiosis establishment and growth of three micropropagated species
Journal of the American Society for Horticultural Science **118**(6): 896–901

Research findings

VA Mycorrhizae in relation to growth of different tea varieties

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Introduction

The potential of vesicular-arbuscular mycorrhizal fungi in the field of agriculture is well documented. It has been established that VAM fungi increase plant growth through enhanced nutrient uptake and cycling of phosphorus, nitrogen, carbon, zinc, copper and other minerals.

Despite the ubiquitous occurrence of VA fungi in diverse groups of soils and plant species, information on mycorrhizal association with the tea plant is very limited (Sieverding and Toro, 1987; Morita and Konishi, 1989; Mridha, Begum and Begum 1995; Kumaran and Santhanakrishnan, 1995).

Several tea varieties collected from three different tea research stations viz. Tocklai Experimental Station, Jorhat, Assam; Tea Research Station, Kurseong, Darjeeling and UPASI Tea Research Station, Valparai, Tamil Nadu are maintained in the Tea Germplasm Bank in North Bengal University, Siliguri. The performance of these varieties is not uniform despite all conditions being the same. It was, therefore, considered worthwhile to study mycorrhizal association with these tea varieties in relation to their growth.

Material and methods

The following 8 varieties of tea were selected for the present investigation and were categorized into two sections (A and B) on the basis of age (Table 1). Section A included plants (4 varieties) 6 years of age and Section B included plants (4 varieties), 4 years of age and one variety 3 years old.

Tocklai : TV–23, TV–26, TV–29; Teen Ali–17
Darjeeling : B–777
UPASI : UPASI–2, UPASI–9, UPASI–26

Five plants of the same age group in each variety and five samples (rhizosphere soil and roots) of each plant were collected from the Germplasm Bank and brought to the laboratory in tagged polyethylene bags. The rhizosphere soil samples were processed to screen the VAM spore population by the wet sieving and decantation method (Gerdemann and Nicolson, 1963). The root samples were examined following the method of Phillips and Hayman (1970). VAM species were identified by the manual of Schenck and Perez (1987). The growth performance of the plant was categorized on the basis of the foliage borne by them, into good (more than 15), poor (6–10), and very poor (less than 6).

Results and discussion

The average results of five replicates are presented in Table 1. A good correlation could be established between VAM population root colonization and

Table 1 Spore population and root colonization in relation to growth performance of tea varieties

	Variety of tea	*Age of plant* (in years)	*Plant growth condition**	*Spore population** (spores/10 g dry soil)	*Root colonization** (%)
Section A	TV–23	06	Good	30	100
	TV–26	06	Good	15	100
	B–777	06	Poor	15	75
	Teen Ali–17	06	Poor	10	50
Section B	UPASI–2	04	Good	30	100
	UPASI–26	04	Good	20	80
	UPASI–9	04	Good	07	34
	TV–29	04	Good	05	15
	TV–26	03	Very poor	05	35

* date represents average of five replicates

plant growth for the tea varieties of Section A. 100% root colonization and 15–30 spores/10 g dry soil were observed in TV–23 and TV–26, where the growth of individual plants was also good. Teen Ali–17 and B–777 varieties which attained poor growth had 50–75% root colonization and 10–15 spores/10 g dry soil. However, in Section B, 80–100% root colonization and 20–30 spores/10 g dry soil were observed in UPASI–2 and UPASI–26 where growth was good. Plant growth was good in other varieties (TV–29, UPASI–9) but they had only 15–34% root colonization and 5–7 spores/10 g dry soil.

The six-year old TV–26 variety had 100% root colonization and good growth whereas 3-year old plants of the same variety showed only 35% root colonization and very poor growth. It appears that older plants provide adequate carbohydrates to the symbiont for their proliferation and in turn it supplements nutrients for good growth of the plant. *Glomus macrocarpum*, *G. fasciculatum* and *G. ambisporum* were invariably recorded in association with roots of all the varieties whereas the distribution of *G. aggregatum*, *Gigaspora margarita*, *Sclerocystis* and *Scutellospora* was not uniform. *Gigaspora margarita* along with *Glomus* species was found to associate with plants showing good growth.

The positive impact of VA mycorrhizae on plant growth has also been reported by several workers including Krishna, Shetty, Dart et al. (1985), Singh and Subba Rao (1987), Ratti and Janardan, 1996). Sieverding and Toro (1987) also observed the impact of different isolates of VAM fungi on tea and found a three-fold increase in growth parameters compared to non-mycorrhizal plants. Moreover, other factors, such as diverse microbial populations in rhizophere soil or edaphic conditions, also exert a good or bad impact on plant growth and VAM colonization (Finley, 1985; Reid, Parker, Mitchell et al. 1988; Klyuchnikov and Gelster, 1990; Lussenhop, 1996). In case of TV–29 and UPASI–9 (Section B) where plant growth was good despite very poor root colonization by VAM fungi, the viability potential of plants and the growth promoting efficacy of the colonized VAM fungi might be one of the possible reasons but further investigation is required.

References

Finley R D. 1985
Interaction between soil micro-arthropods and endomycorrhizal associations of higher plants
In *Ecological interaction in soil, plants, microbes and animals*, pp. 319–331, edited by A M Fitter, D Atkinson D, D S Read, M B Usher
Oxford, UK: Blackwell Scientific Publications

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

Klyuchnikov A A and Gelster Yu G. 1990
The effects of soil bacteria and amoebas on endomycorrhiza-forming fungus *Glomus mosseae* under condition of saprotrophic growth
Moscow University Soil Science Bulletin **45**(3): 50–52

Krishna, K R, Shetty K G, Dart P J and Andrews D J. 1985
Genotype-dependent variation in mycorrhizal colonization and response to inoculation of pearl millet *Pennisetum americanum*
Plant and Soil **86**(1): 113–26

Kumaran and Santhanakrishnan. 1995
VA Mycorrhiza fungi in tea (*Camellia sinensis*) soil
In *Mycorrhizae: Biofertilizers for the Future*, pp. 33–37, edited by A Adholeya and S Singh
New Delhi: Tata Energy Research Institute. 548 pp.
[Proceedings of the Third National Conference on Mycorrhiza, New Delhi, India, 13–15 March 1995]

Lussenhop J. 1996
Collembola as mediators of microbial symbiont effects upon soybean
Soil Biology and Biochemistry **28**(3): 363–369

Morita and Konishi. 1989
Relationship between VA mycorrhizal infection and soil phosphorus concentration in tea fields
Soil Science and Plant Nutrition **35**(1): 139–143

Mridha M A U, Begum N and Begum F. 1995
Studies on VA mycorrhizal fungi of tea and associated legumes
In *Mycorrhizae: Biofertilizers for the Future*, pp. 8–11, edited by A Adholeya and S Singh
New Delhi: Tata Energy Research Institute. 548 pp.
[Proceedings of the Third National Conference on Mycorrhiza, New Delhi, India, 13–15 March 1995]

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

Ratti N and Janardhanan K K. 1996
Response of dual inoculation with VAM and *Azospirillum*, on the yield and oil content of Palmarosa (*Cymbopagan martini var-motia*)
Microbiological Research **151**(3): 325–328

Reid R K, Parker W C, Mitchell R J, Garret H E, Henderson G S, and Cox G S. 1988
Vesicular arbuscular mycorrhizal influence on growth and aluminium tolerance of sugar maple seedlings
In *Proceedings of 10th North American Forest Biology Workshop, Physiology and Genetics of Reforestation*, pp. 308–315
Vancouver: University of British Columbia
[Vancouver, British Columbia, 10–22 July1988]

Schenck N.A. and Perez Y. 1987
Manual for the identification of VA mycorrhizal fungi INVAM
Gainseville, USA: University of Florida. 245 pp.

Sieverding E and Toro S T. 1987
Growth of coffee and tea plants in nurseries inoculated with different VAM fungal species
In *Proceedings of the Seventh North American Conference on Mycorrhiza*, edited by D M Sylvia, L L Hung, and J H Graham Florida: University of Florida. 364 pp.
[Gainesville, Florida, 3–8 May 1987]

Singh C S and Subba Rao N S. 1987
Yield and phosphorous content of wheat (*Triticum aestivum*) as influenced by co-inoculation with *Azospirillum brasilense* and *Glomus fasiculatum*
Mycorrhiza Round Table: *Proceedings of a workshop,* pp. 335–346, edited by Verma A K, Oka AK, Mukerji K G, Tilak K V B R, Janak Raj.
New Delhi: India
[JNU-IDRC, Canada sponsored National Workshop on Mycorrhizae, New Delhi, India, 13–15 March 1987]

Technology for mass-multiplication of arbuscular mycorrhizal (AM) fungi for field inoculation to sweet potato

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Introduction

AMF (arbuscular mycorrhizal fungi) form symbiotic associations with most tropical crop plants. These fungi can improve plant growth under conditions of low fertility, confer resistance/tolerance to some pathogens, improve the water balance of the plants and contribute to the formation of soil structure (Hayman 1982). Mass inoculation of AMF with efficient strains in the field for cultivated crops has usually met with little success because of the inability to culture the fungus axenically. Large-scale production of AM inoculum for field inoculation is technically feasible using the roots of host plants grown in sterilized soil to produce pot cultures of a single AM species.

Sweet potato (*Ipomoea batatas* (Lam.) L.) is now being recognized as the foremost tuber crop in respect to calorific value. The dependence of the crop on mycorrhiza for growth and nutrient uptake has been well established (Harikumar 1997). In this study an attempt has been made to develop a technology for the mass multiplication and mass inoculation of AMF to field-grown sweet potato.

Materials and methods

Cassava preinoculated with AMF, *Glomus microcarpum*, was used as the source of AMF. Cassava skin containing spores ranging from 30 to 40/ cm^2 (wet weight basis) was cut into small pieces and mixed with lignite slurry (2 parts of lignite having 40% moisture). The mixture was spread uniformly in a layer above sterile soil in the pot and was covered with a top layer of soil.

Sweet potato (var. Kanjangad) vine cuttings (30 cm length) were collected from healthy plants, stacked and kept for 3 days for defoliation. Mycorrhizal inoculation was done batch-wise by planting the stacks in pots containing inoculum (Figure 1) at two fertility levels of phosphorus, high (140 ppm) and low (69.75 ppm). Root samples were collected at intervals of ten days up to 40 days after inoculation (DAI) and processed for monitoring mycorrhizal infection (Giovannetti and Mosse 1980). Inoculum build-up in soil was assessed (Gerdemann and Nicolson 1963) after each growing season of sweet potato.

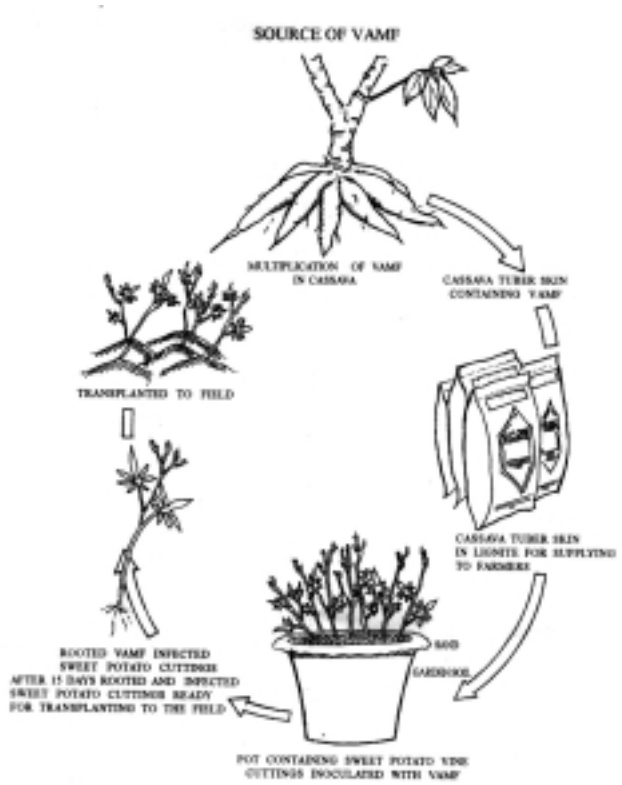

Figure 1 Flow chart showing mass multiplication of VAMF for field inoculation in sweet potato

Results and discussion

Co-inoculation of *G. microcarpum* in the roots of sweet potato vine cuttings was observed from 10 DAI onwards. At low levels of P the percentage of colonization and intensity of infection was greater than at high levels of P. Establishment of the fungus was similar at both low and high levels of P, but the colonization rate was different. From 10 DAI onwards both percentage colonization and intensity of infection showed an upward trend. Even at 40 DAI percentage colonization approximated 100 and 84.61 in roots at both low and high levels of P, respectively (Table 1). The intensity of infection also showed the same trend. Spore density in the soil was found to increase after continuous cultivation for 3–4 seasons (Table 2).

The technique developed in the present study for mass multiplication and mass inoculation of AMF needs no sophisticated equipment or skill. Peels of cassava skin from the tubers which harbour AMF are often left waste (Potty 1985) and can effectively be utilized as inoculum for further multiplication/propagation. Repeated mass inoculation over 2–3 years can effectively build up sufficient mycorrhizal population in the field reducing the need for further inoculation. Even a farmer with little education can successfully practise this technology in the field.

Table 1 Establishment of *G. microcarpum* in rooted sweet potato vine cuttings at two levels of P

AMF	Fungal characters	P levels	DAI 10	20	30	40
G. microcarpum	Percentage colonization	Low	40	90.48	94.73	100
		High	36	73.33	85.71	84.61
	Intensity of infection	Low	16	30.53	40.91	53.85
		High	15.24	28.53	36.67	52.67

Mean of 10 replications

Table 2 Inoculum build-up in sweet potato soils after continuous cultivation

P levels	Spore load/50 g soil* Season I	Season II	Season III
High	78	116	206
Low	102	133	274

* Average of 10 replications

References

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

Giovannetti M and Mosse B. 1980
An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots
The New Phytologist **84**: 489–500

Harikumar V S. 1997
Endomycorrhizal studies in sweet potato
Thiruvananthapuram: University of Kerala.
[Doctoral thesis]

Hayman D S. 1982
Influence of soils and fertility on activity and survival of vesicular arbuscular mycorrhizal fungi
Phytopathology **72**: 1119–1125

Potty V P. 1985
Cassava as an attempt host for multiplication of VAM fungi
Plant and Soil **88**: 135–137

A survey of some cultivated legume crops of Aligarh district to determine the occurrence of AM fungi

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Introduction

There are several reports that AM fungi occur worldwide in diverse habitats (Bagyaraj, Manjunath and Reddy 1979; Parvathi, Venkateshwaralu and Rao 1984). Schmidt and Scow (1986) found that AM fungi were present to varying degrees in the roots of at least some members of all plant communities sampled. Medina, Kretshmer and Sylvia (1988) observed that legume species differ in percentage of root colonization and total spore density among sites. AM distribution, occurrence and association were studied at both cultivated (Rao, Pawar and Singh 1990) as well as uncultivated sites (Blaszkowski, 1993). Level of AM colonization varied between the two crops from site to site and species to species and was not related to soil properties (Talukdar and Germida, 1993; Muthukumar, Udaiyan and Manian 1996).

Material and methods

During the year 1995-96, sampling was carried out separately for each crop in fields of leguminous crops at polluted sites (Javan and Harduaganj) and pollution-free sites (Kwarsi and Kharaishwar). Six commonly cultivated leguminous crops, pea, chickpea, pigeonpea, cowpea, mungbean and clover were selected for study at each site and their characteristics are given in Tables 1a and 1b. Fifty samples collected randomly were thoroughly mixed. Seven root samples of each plant species were collected at random to measure root colonization of AM fungi.

Spores of AM fungi were isolated by the wet sieving and decanting method (Gerdemann and Nicolson, 1963) and the clearing and staining of the roots was done by the method of Phillips and Hayman (1970). Percentage of root colonization was determined by the slide method (Giovannetti and Mosse, 1980).

$$\% \text{ AM association} = \frac{\text{Number of mycorrhizal segments}}{\text{Total number of segments screened}} \times 100$$

Isolated spores were identified using keys provided by Hall and Fish, 1979.

Results

The data in Table 2 indicate that the AM population was affected by pollutants released from the Kasimpur thermal power station. It is clear that pollutant-free sites always yielded higher populations of AM spores compared to polluted sites. Among the different crops, clover supported the highest production of spores while cowpea yielded the minimum in all the four sites investigated.

At the polluted sites Harduaganj and Javan, mung bean showed the second highest number of AM spores followed by chickpea, pea and pigeonpea. Statistical analysis of the data shows that the AM spore number varied significantly between crops.

At pollution-free sites Kharaishwar and Kwarsi, the highest and lowest spore numbers were recorded in clover and cowpea, respectively. The pigeonpea stood close to cowpea in spore number, varying only insignificantly from that of cowpea.

Table 1a Soil characteristics at the polluted sites

		Particle size %					*Available nutrients* (kg/ha)			
	Soil texture	*Sand*	*Silt*	*Clay*	*Organic Carbon* (%)	*pH*	*N*	*P*	*K*	*Distance from Kasimpur power station* (km)
Javan	Sandy loam	74.5	19.4	6.1	0.623	8.5	134	8.2	94.5	7
Harduaganj	Sandy loam	75.0	20.2	4.8	0.775	8.7	147	8.5	90.9	4

Table 1b Soil characteristics at the non-polluted sites

		Particle size %					*Available nutrients* (kg/ha)			
	Soil texture	*Sand*	*Silt*	*Clay*	*Organic Carbon (%)*	*pH*	*N*	*P*	*K*	*Distance from Kasimpur power station* (km)
Kharaishwar	Sandy loam	70.0	17.8	12.2	0.325	7.7	101	7.4	97.9	18
Kwarsi	Sandy loam	76.1	16.2	7.2	0.450	8.0	120	7.7	95.8	13

Table 2 Total AMF spore-count at different sites under different crops

	Non-polluted sites		*Polluted sites*	
Crops	*Kharaishwar*	*Kwarsi*	*Javan*	*Harduaganj*
Pea	364	342	215	200
Chickpea	367	348	225	210
Pigeonpea	355	335	205	195
Cowpea	345	323	190	175
Mung bean	374	345	240	220
Clover	405	388	310	295
C D at 5%	12	15	9	8

The second highest number of spores was recorded in mungbean followed by chickpea and pea. Analysis, however, showed that the difference in spore number between the above crops was not statistically significant.

The results shown in Table 3 indicate that the percentage colonization of AMF was affected by pollutants. It is also evident that the per cent colonization in clover was maximum, 60-64% and 70-74%, at the polluted and non-polluted sites respectively. In mung bean, the colonization percentage was the second highest of all the four sites with an average of 44%. In pea and pigeonpea, the average percentage of root colonization was 54% and 48.5%, respectively; while in chickpea it was 56.5%.

Table 3 Mycorrhization at different sites under different crops

	Non-polluted sites		*Polluted sites*	
Crops	*Kharaishwar*	*Kwarsi*	*Javan*	*Harduaganj*
Pea	62	58	50	46
Chickpea	60	67	54	48
Pigeonpea	53	55	45	41
Cowpea	47	50	40	39
Mung bean	70	63	58	55
Clover	74	70	64	60
C D at 5%	4	5	4	4

Discussion

The present investigation shows that all leguminous crops are mycorrhizal as reported by Mosse (1976). The total number of AM spores were highest in clover fields indicating that they supported better multiplication compared with other crops at polluted as well as non-polluted sites (Paulitz and Linderman, 1989).

Acknowledgments

The first author is grateful to the Aligarh Muslim University, Aligarh for providing financial assistance during the course of investigation.

References

Bagyaraj D J, Manjunath A and Reddy D D R. 1979
Interaction of vesicular-arbuscular mycorrhiza with root-knot nematode in tomato
Plant and Soil **51**:397–403

Blaszokowski J. 1993
Comparative studies on the occurrence of arbuscular fungi and mycorrhizal (Glomus) in cultivated and uncultivated soil of Poland
Acta Mycologia **28**:93–140

Gerdemann J W and T H Nicolson 1963
Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting
Transactions of the British Mycological Society **46**: 235–246

Giovannetti M and Mosse B. 1980
An evaluation of technique for measuring vesicular-arbuscular mycorrhizal infection in roots
The New Phytologist **84**: 498–500

Hall I R and Fish B J. 1979
A key to endogonaceae
Transactions of the British Mycological Society **73**: 261–270

Medina O A, Kretshmer Jr, A E, and Sylvia D M.1988
The occurrence of VA mycorrhizal fungi on tropical forage legumes in South Florida
Tropical Grasslands **22**:73–78

Mosse, B. 1976
In *Rhizobium symbiosis in Tropical Agriculture*, pp. 275–280, edited by J M Vicent, A S Whitney and E Bose Honolulu: University of Hawaii

Muthukumar T, Udaiyan K, and Manian S. 1996
Vesicular-arbuscular mycorrhizae in tropical sedges of southern India
Biology and Fertility of Soils **22**: 96–100

Parvathi K, Venkateshwaralu K, and Rao A S. 1984
Occurrence of VA mycorrhizae on different legumes in laterite soil.
Current Science **53**: 1254

Paulitz T C and Linderman R G. 1989
Interactions between fluorescent pseudomonads and VA mycorrhizal fungi
The New Phytologist **113**: 37–45

Phillips J M and Hayman D S. 1970
Improved procedures for clearing roots and obtaining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection
Transactions of the British Mycological Society **55**: 158–161

Rao V P, Pawar S E, and Singh S N. 1990
Distribution and intensity of native VAM in Maharashtra region

In *Current Trends in Mycorrhiza Research,* pp. 230–232, edited by B L Jalali and H Chand
Hisar: Haryana Agricultural University. 210 pp.
[Proceedings of the National Conference on Mycorrhiza, Hisar, India, 14–16 February 1990]

Schmidt S K and Scow K M. 1986
Mycorrhizal fungi on the Galapagos Islands
Biotropica **18**: 236–240

Talukdar N C and Germida J J. 1983
Occurrence and isolation of vesicular-arbuscular mycorrhizae on cropped field soils of Saskatchewan, Canada
Canadian Journal of Microbiology **39**: 567–575

Changes in the root development pattern of bamboo and sweet orange plants upon arbuscular mycorrhization

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Introduction

The influx of phosphorus in plant roots colonized by arbuscular mycorrhizal fungi can be 3-5 times higher than that in non-mycorrhizal roots (Schachtman, Reid and Ayling 1998). The underlying mechanism is commonly explained by the efficiency with which mycorrhizal roots, with the external hyphae extending beyond the depletion zone surrounding the root and its hairs, exploit the soil for acquisition of nutrients (Smith and Gianinazzi-Pearson, 1988). Whether the AM fungi in symbiosis function only as appendages to existing roots or alter the architecture of the host root system in a significant way to allow better capture of nutrients has received some attention recently (Koide and Schreiner 1992; Tisserant, Gianinazzi and Gianinazzi-Pearson 1996). Any such change in root developmental pattern assumes significance in understanding the molecular basis of morphogenesis of functional mycorrhiza also (Barker, Tagu, and Delp 1998). We present here evidence to show that root development patterns of two AM-responsive plants – *Dendrocalamus strictus* (bamboo), a monocot, and *Citrus sinensis* (sweet orange), a dicot – are significantly changed due to AM-infection in a low nutrient soil, having implications for possible improvements in nutrient acquisition efficiency.

Materials and methods

Seedling plants of *Citrus sinensis* and *Dendrocalamus strictus* were grown in pot culture in a low nutrient laterite soil, with or without AM inoculation, in a glasshouse under ambient environment. The sandy loam soil had the following properties: pH 6.0, organic carbon 0.27%, total nitrogen 0.03%, available (Olsen's) phosphorus 5 ppm, and available potassium 13.5 ppm. AM infectivity potential of the solarized soil was raised from less than 5 to ca. 100 infective propagules per gram by adding maize root-based mixed AM species inoculum before planting 50-day old *D. strictus* bamboo and nucellar seedlings of sweet orange, raised separately in sterile sand. AM-infected maize root inoculum was produced in a sterile sand-soil mixture inoculated with surface-sterilized (Watrud, 1984) *Glomus mosseae, G. fasciculatum* and *Gigaspora margarita* spores isolated from rhizosphere soils of common plants from a similar location. Observations of root development characters were recorded by macro- and micrometric measurements of the whole root system of replicate plants harvested 300 days after planting.

Results

Dendrocalamus strictus

D. strictus is a moderately arbuscular mycorrhiza responsive (Bhattacharya, Saha, Banerjee et al. 1995) bamboo plant. Thick (400–800 μm dia), downward-growing primary roots are produced from the root primordia of its pachymorph rhizomes. The primary roots are not infected by the AM fungi. Progressively finer successive series of lateral branches are produced from the primary roots and are identified as 1st, 2nd, and 3rd order laterals. These laterals spread somewhat horizontally and together form a network of fine roots, 8–40 cm below the surface (Figure 1a, b). Root hairs are few and short (<2 mm), present mostly on the third order laterals. Mycorrhizal presence is restricted to the laterals, more in the 3rd than 2nd order and rarely in the 1st order branches. Mycorrhization resulted into a significant increase in the lateral root development of *D. strictus* plants, particularly with respect to the number of 3rd order branches per cm of primary root (Table 1). The total number of lateral roots per cm of primary root was significantly higher in mycorrhizal than non-mycorrhizal plants, the difference being individually significant for the 3rd order laterals only (Table 1). While average lengths of the lateral roots of any of the orders individually did not change

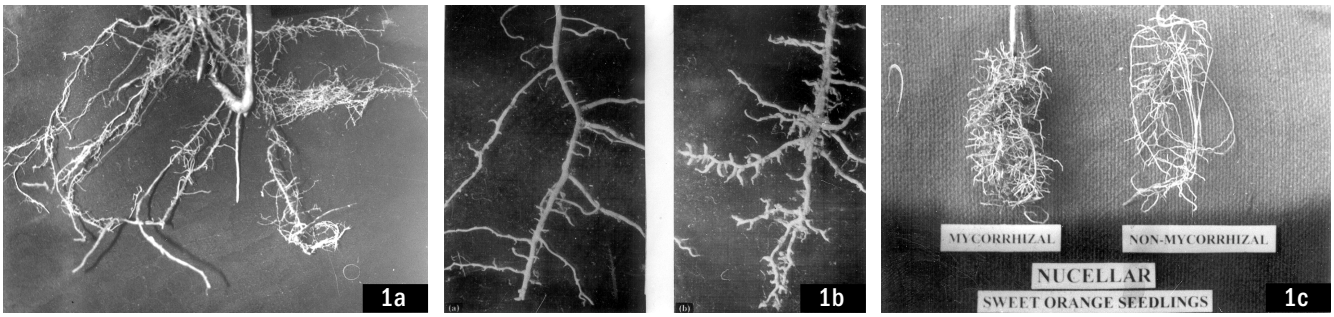

Figure 1 Changes in root development pattern of sweet orange and bamboo plants upon mycorrhization

Table 1 Branching intensity and surface area of different categories of lateral roots of *Dendrocalamus strictus* bamboo

Categories	*NM*	*M*	*'t'* $_{0.05}$
Number of branches per cm of primary root			
1st order	8.0 ± 1.7	7.0 ± 1.7	0.71
2nd order	13.7 ± 1.5	20.7 ± 4.7	2.44
3rd order	83.7 ± 4.0	196.0±18.1	10.50*
Total	105.3 ± 4.0	223.7±17.0	11.70*
Average length			
1st order (cm)	0.98 ± 0.14	0.98 ± 0.04	0.08
2nd order (cm)	0.41 ± 0.05	0.41 ± 0.16	0.03
3rd order (mm)	0.60 ± 0.09	0.69 ± 0.04	1.54
Total length traversed by branches per cm of primary root (cm)			
1st order	8.0 ± 0.83	6.79 ± 1.47	1.24
2nd order	5.60 ± 0.27	8.11 ± 2.06	2.09
3rd order	5.04 ± 0.77	13.57 ±1.22	10.28*
Total	18.65 ± 2.86	28.46 ± 3.23	3.94*
Diameter (mm)			
1st order	0.36 ± 0.07	0.54 ± 0.17	1.67
2nd order	0.19 ± 0.04	0.23 ± 0.08	0.75
3rd order	0.11 ± 0.03	0.13 ± 0.03	0.89
Surface area (sq mm)			
1st order	89.43 ± 9.27	114.53 ± 24.72	1.64
2nd order	34.30 ± 1.61	59.10 ± 15.00	2.84*
3rd order	17.10 ± 2.62	56.26 ± 5.00	12.00*

Table 't' value $_{0.05, d.f\ 4}$ = 2.776, * Significant

significantly upon mycorrhization, the total length traversed by the laterals per cm of primary root, particularly the 3rd order laterals, increased significantly in mycorrhizal over non-mycorrhizal plants. Diameters of the mycorrhizal plant root laterals also showed a marginal increase over non-mycorrhizal plant root laterals. Due to increases in their numbers and hence total length, the total surface area of the 3rd and also the 2nd order laterals increased in mycorrhizal over non-mycorrhizal plants. Frequency distribution data of the different size groups of lateral roots showed that the increase in the mean number of 3rd order laterals in mycorrhizal plants was almost exclusively due to the very significant increase in the number of smaller laterals (Figure 2). Increased lateral root developmental activity of *D. strictus* plants upon mycorrhization by production of a significantly higher number of short 3rd order laterals, resulting into increased absorptive lateral root surface area was indicated by the data.

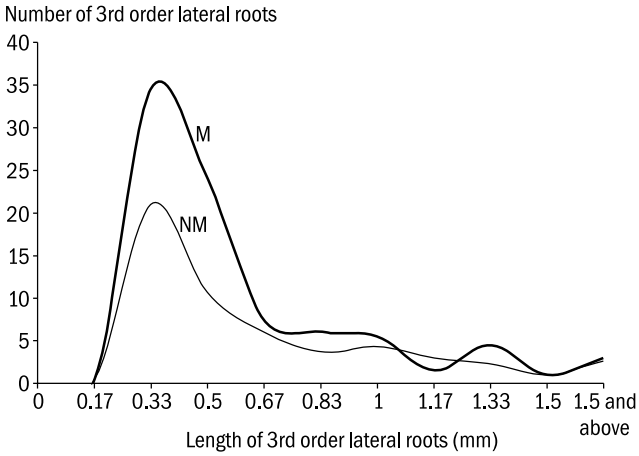

Figure 2 Frequency distribution data of 3rd order lateral roots

Citrus sinensis

C. sinensis, a highly mycorrhiza-dependent plant (Menge, Johnson, and Platt 1978), produces progressively finer 1st to 3rd order lateral branches from the taproot. The laterals are devoid of root hairs and are infected by the AM fungi. Presence of mycorrhizal elements is higher in 3rd, than 2nd and 1st order laterals.

Taproots of mycorrhizal plants showed a significant increase in length and weight compared to the taproots of non-mycorrhizal plants although there were no mycorrhizal elements in these roots (Table 2). Significantly, the taproot weight per unit length of mycorrhizal plants also increased. Number of lateral roots, both total per plant and per unit length of taproot, increased significantly in mycorrhizal over non-mycorrhizal plants (Table 3).

Table 2 Development of tap root of mycorrhizal and non-mycorrhizal sweet orange plants

	NM	*M*	*'t' 0.05*
Average tap root length per plant (cm)	5.2 ± 1.4	8.9 ± 1.4	5.15
Average tap root weight per plant (mg)	92.0 ± 36	270.0 ± 53	7.98
Average tap root weight per cm (mg)	17.4 ± 4.2	30.3 ± 3.3	6.42

Table 't' value $_{0.05, d.f\ 14}$ = 2.145

Average lengths of lateral roots of mycorrhizal plants, of all orders were significantly lower than those of the non-mycorrhizal plants (Table 4). But, due to the increase in number the total length traversed by the mycorrhizal plant lateral roots was higher than that of the non-mycorrhizal plants. The differences in lateral root length, between mycorrhizal and non-mycorrhizal plants, were significant for the 1st and 2nd order laterals, but not for the 3rd order laterals, owing to the extreme reduction in their size. Total length of individual order lateral roots per cm of taproot, however, did not differ significantly between mycorrhizal and non-mycorrhizal plants. Frequency distribution data of the different size groups of lateral roots showed that the observed significant increase in their mean number was due to the significant increase in the number of short sized laterals (Figure 3). Pronounced lateral root initiation activity associated with reduced apical growth of the individual branches was indicated by the data (Figure 1c).

Discussion

Roots are the sites of infection of arbuscular mycorrhizal fungi, but, surprisingly, the effects of mycorrhiza have been examined and interpreted far less than shoots. The pronounced cytoskeletal changes that occur in host cells due to intra-and intercellular colonization of the biotrophic AM fungus (Bonfante and Perotto, 1995), is expected to be less than innocuous for morphogenesis and development of roots. Although, due to overall

Table 4 Average length (cm) of lateral roots of non-mycorrhizal and mycorrhizal sweet orange plants

	NM	M	't' 0.05
1st order	5.10 ± 1.25	2.91 ± 0.32	2.94
2nd order	2.02 ± 0.16	1.33 ± 0.21	4.56
3rd order	1.65 ± 0.17	0.87 ± 0.25	4.45

Table 't' value $_{0.05, d.f\ 4}$ = 2.776

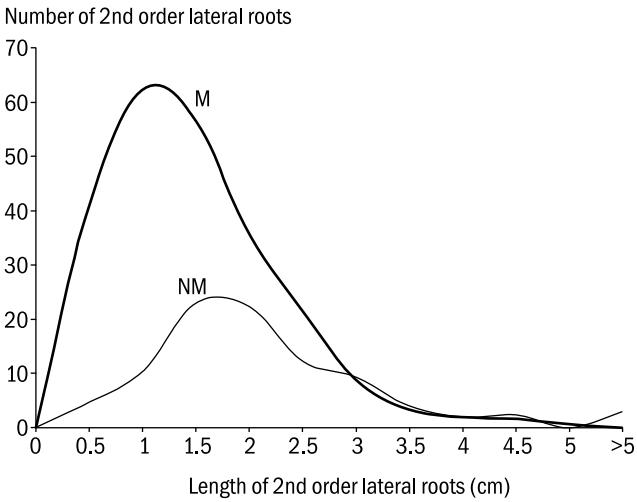

Figure 3 Frequency distribution data of 2nd order lateral roots

Table 3 Development of different order lateral roots of non-mycorrhizal and mycorrhizal sweet orange plants

	Per plant			*Per cm of tap root*		
	NM	*M*	*'t' 0.05*	*NM*	*M*	*'t' 0.05*
Number of lateral roots						
1st order	17.5 ± 0.5	48.3 ± 3.5	15.1	3.4 ± 0.1	5.4 ± 0.4	8.8
2nd order	84.5 ± 3.5	230.0 ± 37.0	6.8	16.3 ± 0.7	25.8 ± 4.2	3.9
3rd order	49.67 ± 6.51	128.0 ± 19.3	6.7	9.5 ± 1.2	14.4 ± 2.2	3.4
Total	151.7 ± 9.5	406.3 ± 58.9	7.4	29.2 ± 1.8	45.6 ± 6.6	4.16
Total length traversed by the lateral roots (cm)						
1st order	89.2 ± 2.5	140.6 ± 10.2	8.5	17.2 ± 0.5	15.8 ± 1.2	1.9
2nd order	169.0 ± 7.0	305.9 ± 49.3	4.8	32.8 ± 1.4	34.4 ± 5.5	0.6
3rd order	81.9 ± 10.7	111.4 ± 16.8	2.6	15.8 ± 2.1	12.5 ± 1.9	2.0
Total	337.8 ± 15.4	554.2 ± 75.0	4.9	65.7 ± 3.4	62.7 ± 8.4	0.6

Table 't' value $_{0.05, d.f\ 4}$ = 2.776

improvements in nutrition and metabolism there may be an absolute increase of root mass in mycorrhizal over non-mycorrhizal plants, a decrease in the root:shoot ratio is the typical arbuscular mycorrhizal response of most plants dependent on mycorrhiza physiologically (Smith and Gianinazzi-Pearson, 1988). However, a decrease in absolute root mass development may also occur due to sink competition for photosynthate between the partners of symbiosis, in relatively mycorrhiza independent or non-mycotrophic plants (Marschner, 1995). Apart from such gross changes in root mass development (total root length, weight, volume etc.), changes in the fine structure of roots (branch number, length, fineness, root hair production etc.) have sometimes been reported (Koide, 1991). More significant in the context of plant nutrition may be the possible changes in root geometry or branching pattern, modifying the whole root architecture, as seen here and suggested earlier also (Berta, Fusconi, Trotta et al. 1990). Evidence is now available to postulate that such structural changes in root development may be the cause (Barker, Tagu and Delp 1998), rather than the effect (Koide, 1991), of improved phosphorus nutrition.

In the two contrasting plant species, bamboo and sweet orange, mycorrhiza-induced increased lateral root development activity resulted in increased lateral root number, branch length, and surface area per unit length of the non-feeding, anchoring roots. Such changes in lateral root development activity, were more pronounced for the more mycorrhiza-responsive dicot citrus plant, but the pattern of changes was similar at a gross level in the less responsive monocot bamboo plant also. Characteristically, in the highly responsive citrus plant, the lateral root branching effect was operative some distance away from the main site of colonization. In the less responsive bamboo plant, the effect was restricted to the main site of colonization only. Apart from the gross changes in rooting density, the changed root geometry of the mycorrhizal plants (large number of short feeding roots per unit primary root length) would provide for changed root architecture for a more efficient exploration of a larger volume of soil. The same, along with the extramatrical hyphae would function in more efficient acquisition of nutrients from the soil and add to the physiological efficiency of mycorrhizal plants in nutrient-deficient soils. The large number of short root laterals due to mycorrhization can also add to the 'escape' competence of plants against root pathogens.

References

Barker S J, Tagu D, and Delp G. 1998
Regulation of root and fungal morphogenesis in mycorrhiza symbiosis
Plant Physiology **116**(4): 1201–1207

Berta G, Fusconi A, Trotta A, and Scannerini S. 1990
Morphogenetic modifications induced by the mycorrhizal fungus *Glomus* strain E_3 in the root system of *Allium porrum* L.
The New Phytologist **114**(2): 207–215

Bhattacharya P M, Saha J, Banerjee K, Ali M and Chaudhuri S. 1995
Mycorrhizal relations of bamboo species in different soil situations of West Bengal
In *Mycorrhizae: biofertilizers for the future*, pp. 17–21, edited by A Adholeya and S Singh
New Delhi: Tata Energy Research Institute. 548 pp.
[Proceedings of the Third National Conference on Mycorrhiza, New Delhi, India, 13–15 March 1995]

Bonfanto P and Perotto S. 1995
Strategies of arbuscular mycorrhizal fungi when infecting host plants
The New Phytologist **130**(1): 3–21

Koide R T and Schreiner R P. 1992
Regulation of vesicular-arbuscular mycorrhizal symbiosis
Annual Reviews of Plant Physiology and Plant Molecular Biology **43**: 557–581

Koide R T. 1991.
Nutrient supply, nutrient demands and plant response to mycorrhizal infection
The New Phytologist **117**(3): 365–386

Marschner H. 1995
Mineral nutrition of higher plants
New York: Academic Press. 889 pp.

Menge J A, Johnson E L V, and Platt R G. 1978
Mycorrhizal dependency of several citrus cultivars under three nutrient regimes
The New Phytologist **81**: 553–559

Schachtman D P, Reid R J, and Ayling S M. 1998
Phosphorus uptake by plants: From soil to cell
Plant Physiology **116**: 447–453

Smith S E and Gianinazzi-Pearson, V. 1988
Physiological interactions between symbionts in vesicular arbuscular mycorrhizal plants
Annual Reviews of Plant Physiology and Plant Molecular Biology **39**: 221–244

Tisserant B, Gianinazzi S, and Gianinazzi-Pearson V. 1996
Relationships between lateral root order, arbuscular mycorrhiza development, and the physiological state of the symbiotic fungus in *Platanus acerifolia*
Canadian Journal of Botany **74**(12): 1947–1955

Watrud L S. 1984
Spore germination of vesicular-arbuscular mycorrhizal fungi
In *Methods and Principles of Mycorrhizal Research*, pp. 81–83, edited by N C Schenck
Minnesota, USA: American Phytopatholgical Society. 244 pp.

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.

- *Mycorrhiza*
- *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]
Rincon A, Alvarez I F and Pera J*. 2001	**Inoculation of containerized *Pinus pinea* L. seedlings with seven ectomycorrhizal fungi** *Mycorrhiza* **11**(6): 265–271 [*Pera J, IRTA, Department of Patologie Vegetal, Ctr Cabrils, Ctra Cabrils S-N, Barcelona 08348, Spain]
Schreiner R P*, Ivors K L and Pinkerton J N. 2001	**Soil solarization reduces arbuscular mycorrhizal fungi as a consequence of weed suppression** *Mycorrhiza* **11**(6): 273–277 [*Schreiner RP, USDA ARS, Horticultural Crops Research Lab, 3420 NW Orchard Ave, Corvallis, OR 97330 USA]
Vierheilig H, AltHug M, Wiemken A, and Boller T. 2001	**Hyphal in vitro growth of the arbuscular mycorrhizal fungus *Glomus mosseae* is affected by chitinase but not by beta-1, 3-glucanase** *Mycorrhiza* **11**(6): 279–282 [*Vierheilig H, Univ Laval, Centre Recherche Biologie Forestiere, Pavillon CE Marchand, Laval, PQ G1K 7P4, Canada]
EgertonWarburton L* and Allen M F. 2001	**Endo- and ectomycorrhizas in *Quercus agrifolia* Nee. (Fagaceae): patterns of root colonization and effects on seedling growth** *Mycorrhiza* **11**(6): 283–290 [*Egerton-Warburton L, Chicago Botanical Garden, 1000 Lake Cook Road, Glencoe, IL 60022 USA]
Balaz M* and Vosatka M. 2001	**A novel inserted membrane technique for studies of mycorrhizal extraradical mycelium** *Mycorrhiza* **11**(6): 291–296 [*Balaz M, Masaryk University, Faculty of Science, Department of Plant Physiology, Kotlarska 2, CS-61137 Brno, Czech Republic]
Marschner P*, Crowley D E and Lieberei R. 2001	**Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize** *Mycorrhiza* **11**(6): 297–302 [*Marschner P, University of Adelaide, Department of Soil and Water, Waite Campus, PMB1, Glen Osmond, SA 5064, Australia]
Carvalho L M*, Cacador I, MartinsLoucao M A. 2001	**Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal).** *Mycorrhiza* **11**(6): 303–309 [*Carvalho L M, Fac Ciencias Lisboa, Department of Biology Vegetal, Centre for Ecology and Biology Vegetal, Block C2, Campo Grande, P-1749016 Lisbon, Portugal]
Bago B*, Zipfel W, Williams R M, Jun J, Arreola R, Lammers P J, Pfeffer P E, ShacharHill Y. 2002	**Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis** *Plant Physiology* **128**(1): 108–124 [*Bago B, Estacion Experimental Zaidin, Departmento Microbiologie Suelo and Sistemas Simbiot, Calle Profesor Albareda 1, E-18008 Granada, Spain]

Centre for Mycorrhizal Culture Collection

Biodiversity of AMF and agricultural potential III: Approaches to study the metabolic status and functional diversity of resident AMFs

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The description of diversity within a natural community or habitat is important for exploiting it. But, diversity at the functional rather than the taxonomic level is important for the long-term stability of an ecosystem.

Functioning refers to those AM (arbuscular mycorrhizal)-specific processes which may influence mineral nutrition, stress tolerance and eventually also the growth of the plant (Jakobsen, 1994).

If resident mycorrhizal fungi are to be managed in field conditions, the keystone protocol is to determine their infectivity and the functional efficiency of these fungi. The infectivity of the mycorrhizal fungi can be defined as the rate and extent of mycorrhiza formation (Abbott and Robson, 1982), and therefore depends on the number of propagules in the soil and the capacity of those propagules to produce infective hypha which reach the root surface. Currently, four types of propagules are recognized: spores, hyphae in dead root fragments, hyphae (and/or vesicles) in living roots and extraradical hyphae. The metabolic status of these propagules governs the infectivity of the fungi. Prediction of infectivity prior to plant and establishment is essential if inoculation with functionally-efficient AMF is to be considered or adjustments in fertilizer applications allowed. Microscopically, it is not always possible to reveal non-viable spores as they can maintain a visually normal appearance in soil for extended periods (McGraw and Hendrix, 1986). The use of vital stains is an alternative method for assessing spore viability (An and Hendrix, 1988; Meier and Chavat, 1993; Walley and Germida, 1995). The tetrazolium salt, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) are used to estimate the viability of AMF spores. Similarly, routine root staining techniques (using trypan blue, acid fuchsin, chlorazol black E) for revealing mycorrhizal structures do not distinguish living from dead fungi. Succinate dehydrogenase, a mitochondrial enzyme, has been used histochemically to detect the proportion of infected roots/hypha in which the fungus is alive (Ocampo and Barea, 1985; Kough and Gianinazzi-Pearson, 1986; Smith and Gianinazzi-Pearson, 1990). But the estimation of AMF spore viability and the succinate dehydrogenase activity method are not indicative of the efficiency of the fungus in terms of plant growth.

The positive role of arbuscular mycorrhizae in the growth of many plant species generally results from a greater efficiency of mycorrhizal roots to take up phosphate, mainly due to the capacity of actively-absorbing external hyphae to better exploit the labile pool of available soil phosphate (Smith and Gianinazzi-Pearson, 1988). Studies of the physiological basis of phosphate absorption, accumulation and translocation by AM fungi indicate that all these processes are metabolically dependent (Gianinazzi-Pearson and Gianinazzi, 1986; Thomson, Clarkson and Brain, 1990). Amongst enzymes, the alkaline phosphatases are known to be involved in the phosphate nutrition of AMF and this enzyme activity also indicates the existence of a functional AM symbiosis (Tisserant, Gianinazzi-Pearson, Gianinazzi et al. 1993).

Conventionally, effects of AM on nutrient transport and growth have been studied by comparing AM-colonized plants with uncolonized controls. This approach is less suited to studying the quantification of processes and identifying constraints in AM functioning, so novel methods were introduced, which use a two-compartment/multi-compartment principle to directly measure nutrient transport by the AM mycelium (Jakobsen, 1994). Special multi-compartment systems were exploited by Schüepp, Miller, and Bodmer (1987) in a study of hyphal spread through different growth substrates and since then hyphal compartments have been utilized in several experiments. This includes the hyphal incorporation of carbon by different AM fungi (Jakobsen and Rosendahl, 1990), differences between AM fungi in total lengths and spread of hyphae (Jakobsen, Abbott and Robson 1992a), the depletion of different soil-P fractions and soil acidification by AM hyphae (Li, George and Marschner 1991), time-course studies of hyphal transport of ^{32}P by different AM fungi (Jakobsen, Abbott and Robson 1992b), hyphal transport of N (Johansen, Jakobsen and

Jensen 1992) and hyphal transport of Cu (Li, Marschner, and George 1991) and Zn (Kothari, Marschner and Römheld 1991). Radioactive (Jakobsen, Abbott and Robson 1992b) and stable isotopes (Ames, Reid, Porter et al. 1983) are the most powerful tools for directly demonstrating nutrient transport by hyphae. The isotopes may either be supplied to the intact hyphal network in solution (Hattingh, Gray and Gerdemann 1973), mixed into soil layers at defined positions from the root compartment (Jakobsen, Abbott and Robson 1992b) or uniformly mixed into the soil of the hyphal compartment (Johansen, Jakobsen and Jensen 1992). The two/three-compartment system also facilitates the measurement of the P uptake of the indigenous AM population from disturbed or undisturbed field soils in controlled growth conditions. Ultimately, these measurements should be carried out in the field. Radiotracers have been widely used for studying the root activity of field-grown crops (Abbott and Fraley, 1991) and Dighton, Mason and Poskitt (1990) measured the P uptake by ectomycorrhizas of birch from an aqueous solution of ^{32}P injected into the soil.

References

Abbott L K, Fraley L Jr. 1991
A review: Radiotracer methods to determine root distribution
Environmental Experimental Botany **31**: 1–10

Abbott L K and Robson A D. 1982
Infectivity of vesicular arbuscular mycorrhizal fungi in agricultural soils
Australian Journal of Agricultural Research **33**: 1049–1059

Ames R N, Reid C P P, Porter L K, and Cambardella C. 1983
Hyphal uptake and transport of nitrogen from two ^{15}N-labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus
The New Phytologist **95**: 381–396

An Z Q and Hendrix J W. 1988
Determining viability of endogonaceous spores with a vital stain
Mycologia **80**: 259–261

Dighton J, Mason P A, and Poskitt J M. 1990
Field use of ^{32}P to measure phosphate uptake by birch mycorrhizas
The New Phytologist **116**: 655–661

Gianinazzi-Pearson V and Gianinazzi S. 1986
The physiology of improved phosphate nutrition in mycorrhizal plants
In *Physiological and Genetic Aspects of Mycorrhizae* pp. 101-111, edited by V Gianinazzi-Pearson and S Gianinazzi
Paris: INRA Press

Hattingh M J, Gray L E, and Gerdemann J W. 1973
Uptake and translocation of ^{32}P-labelled phosphate to onion roots by endomycorrhizal fungi
Soil Science **116**: 383–387

Jakobsen I. 1994
Research approaches to study the functioning of vesicular-arbuscular mycorrhizas in the field
In *Management of mycorrhizas in agriculture, horticulture and forestry* edited by A D Robson, L K Abbott and N Malajczuk, pp. 141–147
Netherlands: Kluwer Academic Publishers

Jakobsen I and Rosendahl L. 1990
Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants
The New Phytologist **115**: 77–83

Jakobsen I, Abbott L K and Robson A D. 1992a
External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots
The New Phytologist **120**: 371–380

Jakobsen I, Abbott L K, and Robson A D. 1992b
External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 2. Hyphal transport of ^{32}P over defined distances
The New Phytologist **120**: 509–516

Johansen A, Jakobsen I, and Jensen E S. 1992
Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N
The New Phytologist **122**: 281–288

Kothari S K, Marschner H, and Römheld V. 1991
Contribution of VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil
Plant and Soil **131**: 177–185

Kough J L and Gianinazzi-Pearson V. 1986
Physiological aspects of VA mycorrhizal hyphae in root tissue and soil
In *Physiological and Genetic Aspects of Mycorrhizae* pp. 223–226, edited by V Gianinazzi-Pearson and S Gianinazzi
Paris: INRA Press

Li X-L, George E, and Marschner H. 1991
Phosphorus depletion and pH decrease at the root-soil and hyphae-soil interfaces of VA mycorrhizal with clover fertilized with ammonium
The New Phytologist **119**: 397–404

Li X–L, Marschner H, and George E. 1991
Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover
Plant and Soil **136**: 49–57

McGraw A-C and Hendrix J W. 1986
Influence of soil fumigation and source of strawberry plants on population densities of spores and infective propagules of endogonaceous mycorrhizal fungi
Plant and Soil **94**: 425–434

Meier R and Chavat I. 1993
Reassessment of tetrazolium bromide as a viability stain for spores of vesicular-arbuscular mycorrhizal fungi
American Journal of Botany **80**: 1007–1015

Ocampo J A and Barea J M. 1985
Effect of carbonate herbicides on VA mycorrhizal infection and plant growth
Plant and Soil **85**: 135–141

Schüepp H, Miller D D, and Bodmer M. 1987
A new technique for monitoring hyphal growth of vesicular-arbuscular mycorrhizal fungi through soil
Transactions of the British Mycological Society **89**: 429–435

Smith S E and Gianinazzi-Pearson V. 1988
Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants
Annual Reviews of Plant Physiology and Plant Molecular Biology **39**: 221–244

Smith S E and Gianinazzi-Pearson V. 1990
Phosphate uptake and vesicular arbuscular activity in mycorrhizal *Allium cepa* L.: Effect of photon irradiance and phosphate nutrition
Australian Journal of Plant Physiology **17**: 177–188

Thomson B D, Clarkson D T, and Brain P. 1990
Kinetics of phosphorus uptake by germ tubes of the vesicular-arbuscular mycorrhizal fungus, *Gigaspora margarita*
New Phytology **116**: 637–643

Tisserant B, Gianinazzi-Pearson V, Gianinazzi S, and Gollotte A. 1993
In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections
Mycologia **97**: 245–250

Walley F L and Germida J J. 1995
Estimating the viability of vesicular-arbuscular mycorrhizae fungal spores using tetrazolium salts as vital stains
Mycologia **87**: 273–279

New approaches

Lyophilization of mycorrhizal fungi

A protocol was developed by Sundari, Prakash, Adholeya (1999) to freeze-dry (lyophilize) vegetative mycelium of ectomycorrhizal fungi. (In: Proceedings of the National Conference on Mycorrhiza. March 5–7, 1999, Gwalior, Madhya Pradesh, India). Out of 15 species tested, 14 responded positively and 12 showed 90%–100% viability. Species of Sclerodermataceae were found difficult to freeze dry and species of Pisolithus were particularly sensitive. Every isolate showed a certain lay period in growth after rehydrating the freeze-dried material which was found to be governed by both the specific protectant used and the species under test. The optimization of the freeze-drying protocol included selection of the candidate fungus, optimizing physiological growth conditions and age, standardization of the protectant type and concentration, optimization of the prefreezing method, freeze-drying run and extent of drying, choice of the rehydrant and the extent of rehydration (Sundari S K, Adholeya A 1999, Biotechnology Techniques 13(7): 491–495).

Freeze-drying of Laccaria fraterna revealed that the culture retained its viability after lyophilization and when subjected to quality assurance tests gave consistent results similar to the non-lyophilized culture indicating the stability of the product. Sundari, Adholeya also observed that physical integrity of freeze-dried cultures was comparable to that of non-lyophilized cultures (Canadian Journal of Microbiology 47(2): 172–177). Inter-and intraspecific variation in morphology, physiology and metabolic rate were maintained after lyophilization. Maintenance of total protein content confirmed metabolic stability. According to the assays of viability, a plating assay and determination of total biomass confirmed stable mitotic activity of the freeze-dried cultures.

FORM IV

(as per Rule 8)

Statement regarding ownership and other particulars about Newsletter: *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 Tata Energy Research Institute 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 Tata Energy Research Institute Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003
5.	Editor's Name: Whether Citizen of India: Address:	Dr Alok Adholeya Yes Tata Energy Research Institute 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:	Tata Energy Research Institute Darbari Seth Block, Habitat Place Lodhi Road, New Delhi – 110 003

I, R K Pachauri, hereby declare that the particulars given above are true to the best of my knowledge and belief.

Signature of Publisher
(Sd/-)
Dr R K Pachauri

Date: 1 April 2002

ISSN 0970-695X Regd No. 49170/89

Forthcoming events

Conferences, congresses, seminars, symposiums, and workshops

UNESCO, **France**
10–14 July 2002

International Workshop on Microbial Genetics
Dr S Kannan, Course Director of the Workshop and Head, Department of Microbiology, Ayya Nadar Janki Ammal College, Sivakasi – 626 124, Tamil Nadu, India

Tel. +91 4562 422 100
E-mail skanmbt2000@yahoo.com, skanmbt@rediffmail.com, vgr_anjac@sancharnet.in

Oslo, **Norway**
11–17 August 2002

IMC 7th International Mycological Congress
IMC7 Congress Secretariat, P O Box 24 Blindern, N-0314 Oslo, Norway

Tel. +47 22 85 46 28 • *E-mail* IMC-7@bio.uio.no
Web site http://www.uio.no/conferences/imc7

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11–17 August 2002

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Congress Canada, Bathurst Street Toronto, ON Canada

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E-mail IHCreg@congresscan
Web site http://www.ihc2002.org/ihc2002/cgi.html

Shijiazhuang, Hebei, **People's Republic of China**
15–19 September 2002

International Conference on Environmentally Sustainable Agriculture for Dry Areas for the 2nd Millennium
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International Rice Congress 2002

Web site http://www.irri.org/IRC2002/introduction.htm

Cairo, **Egypt**
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XVth International Plant Protection Congress
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Printed and published by Dr R K Pachauri on behalf of the Tata Energy Research 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.