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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI was established in 1974. While in the initial period the focus was mainly on documentation and information dissemination activities, research activities in the fields of energy, environment, and sustainable development were initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that 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.

Contents

Mass production of AM Fungi: Part 1

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Production technology

VA-mycorrhizal fungi have great potential for use as biofertilizers in agriculture, floriculture, horticulture and forestry as evident from laboratory, greenhouse and limited field studies by a large number of mycorrhiza scientists. The ultimate objective of laboratory and greenhouse studies is to extend them to the field on a commercial level. The potential of using VA-mycorrhizal fungi on a large scale depends upon, 1, the technique by which axenic VAM fungi are grown; 2, the economic production of a large volume and high quality of inoculant; 3, formulation of VAM inoculant preparations with an extended shelf-life and easy handling characteristics and, 4, development of growth-promoting strains superior to indigenous soil VAM fungi (Hua 1990). Research work by various scientists on the mass production of ectomycorrhizal fungi has been published earlier (Singh 1999). The research work hitherto done on mass production of VA-mycorrhizal fungi is presented here.

I Mass Production Techniques

A. Pot culture inoculum

VA-mycorrhizal fungi, being biotrophs, are difficult to cultivate on synthetic media. For bulk production, the natural choice is to develop a dual culture of host roots and VA-mycorrhizal fungi. There are three prerequisites for obtaining such dual cultures. They are: an efficient VA-mycorrhizal fungus, a suitable host and a suitable substrate.

Selection of efficient VAM fungi

In studies conducted at the Soil Science Department, University of Florida, Gainsville, Florida, USA, on the selection of effective VAM fungi for use in an inoculation programme with out-planted sea oats (*Uniola paniculata*), the authors emphasized that a combination of factors including effectiveness, growth rate in culture and the source of the isolates need to be considered while selecting a VAM fungal isolate for commercial inoculum production (Sylvia and Burks 1988). Efficient mycorrhizal fungi for different plant species are listed by Singh (2001). The potential for production on a commercial scale needs to be explored on the basis of their effectiveness (on a broad spectrum of plant species), growth rates, sporocarp production, spore storage and their resistance to hyperparasitism.

Hua (1990) suggested that the technique of chemical mutagenesis might be applied to the VAM fungi to generate mutations on the chromosome so that axenic growth of fungi on artificial media could be achieved. Chemical mutagenesis has been used extensively and successfully in inducing mutations in bacteria, fungi and plants. It is an important tool in the genetic improvement of bacteria and fungi in industry and agriculture. It has been suggested that chemical mutagenesis in conjunction with proper selection and screening might be an important tool to develop superior strains of VAM fungi (Hua 1990).

Selection of a suitable host

In studies conducted at the Department of Plant Protection, Faculty of Agriculture, University of Jordan, Amman, Jordan, five crops were inoculated with *Glomus mosseae* and grown for 10 weeks to assess mycorrhizal infection and sporulation. For all hosts, the percentage of root length infected by the VAM fungus increased rapidly upto 10 weeks after sowing. The infectivity of root inocula increased with increasing percentage of root length infected with the inoculum for all crops except

* Compiled from TERI database – RIZA

where a large number of mature spores had been produced as in barley (1755 spores). The highest spore number was achieved in the rhizosphere of barley plants, followed by chick pea and beans. The lowest spore number was found in the rhizosphere of corn and okra plants. The type of the crop as well as the harvest date greatly influenced the spore population and the extent of root colonization by *G. mosseae* (Al-Raddad 1995).

In studies conducted at the University of Saskatchewan, Department of Soil Science, Saskatoon, Canada, lentil (*Lens esculenta*), Maize (*Zea mays*) and a hybrid of sorghum (*Sorghum bicolor*) and Sudan Grass (*Sorghum sudanense*) were inoculated with *Glomus clarum* and grown in 2 or 6 kg of soil-sand substrate (1:1) until maturity. All the three crops supported growth and spore production by *G. clarum* but the maize plants yielded the largest number of spores. This reflects either the inherent ability of this VAM fungal strain to sporulate or a favourable interaction (related to spore production) with a maize host crop. In the 2-kg pots, twice the number of spores of *G. clarum* per gram of soil-sand substrate was obtained as compared to the 6-kg pots. Enhanced spore production in smaller pots was related to poor plant growth and, nutrient stress. Plants grown in 2-kg pots were stunted, yielded lesser biomass and exhibited severe symptoms of phosphorus and nitrogen deficiency (Talukdar and Germida 1993).

In studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, G K V K, Bangalore, India, seven hosts, *Panicum maximum*, *Chrysopogon fulvas*, *Themeda triandra*, *Chlorus gayana*, *Brachiaria brizantha*, *Passoalum serobiculatum* and *Eleusine coracana*, were screened against *Glomus fasciculatum* to find a suitable host for mass production of the VAM fungus. *C. gayana* (Rhodes grass) was found to be the best host with the highest percentage of mycorrhizal colonization (94%), sporulation (547 spores per 50 ml substrate) and inoculum potential (1.65×10^7 per g). All mycorrhizal parameters studied were the best in *B. brizantha* (Srinivasa and Bagyaraj 1987).

In studies conducted at the Department of Plant Pathology, University of California, Riverside, California, sudan grass, tomato, asparagus, citrus and soyabean were grown in pots, inoculated with *Glomus epigaeus* and were exposed to three water regimes i.e. pots watered manually, misted or enclosed in plastic to increase humidity. Significantly, more sporocarps were produced in sudan grass watered manually than on any other crop in any other water regime. Growth responses due to *G. epigaeus* occurred in all water regimes but varied with plant species. Of all the species tested, only citrus failed to show a significant growth response (Barbara, Daniels, and Menge 1979).

Studies conducted at the Forest Pathology Division, Forest Research Institute, Dehra Dun, India, showed that Paulownia was an ideal plant species for mass multiplication of VAM fungi as its seedlings in polypots develop massive and dense root systems with extensive branching lateral roots. Also, the species is easily propagated from seed or root-shoot cuttings (Mehrotra 1996).

Selection of a suitable substrate

In studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, G K V K Campus, Bangalore, India, different substrates, perlite, vermiculite, soilrite, soil or a combination in various proportions were compared with the traditional substrate of sand-soil mix (1:1 by volume) to select a suitable for mass multiplication of *Glomus fasciculation* inoculum. The perlite-soil rite mix (1:1 by volume) was found to be the best substrate and recorded the highest percent root colonization, spore production and inoculum potential. All mycorrhizal parameters were found to decrease as the proportion of soil in different sand-soil combinations increased. They were lowest in the 1:3 sand-soil combination (Sreenivasa and Bagyaraj 1987).

Production of pot culture inoculum

The most commonly used VAM inocula in laboratory/greenhouse or field experiments have generally been prepared in pot cultures. A variety of host-substrate combinations has been used. A few such combinations found most effective in the mass production of VAM inoculum are described.

(i) *Perlite-soilrite-Rhodes grass combination*

In studies conducted at the Department of Agricultural Microbiology, University of Agricultural Sciences, G K U K Campus, Bangalore, India. Rhodes grass (*Chlores gayana*) seeds were sown in a perlite-soilrite mix (1:1 by volume) inoculated with *Glomus fasciculatum*. Ruakura plant solution was added (50 ml per pot with 2.5 litre substrate) to the pots once in 2, 4, 6, 8, and 10 days and the plants were harvested after 60 days. Addition of Ruakura nutrient solution once in 8 days was found optimum for the mass production of *G. fasciculatum* (Sreenivasa and Bagyaraj 1988a).

In another study conducted at the above University, the maximum number of infective propagules of *Glomus fasciculatum* was obtained 75 days after sowing seeds of Rhodes grass in 16 cm pots holding 700 g of perlite-soilrite (1:1 by volume) substrate. Pruning reduced the number of mycorrhizal propagules for 4 weeks after which the number increased (Sreenivasa and Bagyaraj 1988b).

In further studies at the above University, mass production of *Glomus fasciculatum* was spores using Rhodes grass in a perlite-soilrite (1:1 by volume) mix was best achieved using ammonium nitrate at 80 ppm nitrogen (Sreenivasa, and Bagyaraj 1990).

In another study at the above University, bone meal (12 P), Mussoorie rock phosphate (18 P), and superphosphate (16 P) at 0, 5, 10, 20, 40, and 80 ppm were tested to select the best form and level of P for mass production of *G. fasciculatum* using Rhodes grass as the host and perlite-soilrite as substrate. Percentage of root colonization, extramatrical chlamydospore number and inoculum potential were highest at 100 ppm P applied as rock phosphate. Of the three forms, superphosphate significantly reduced all the mycorrhizal parameters. Plant P concentration increased with increasing level of P applied to the substrate mix in all the three phosphorus forms. The total P concentration of shoot and root was maximum in plants treated with superphosphate followed by bone meal and rock phosphate (Sreenivasa and Bagyaraj 1989).

In another study conducted at the above University, in addition to perlite-soilrite mix as substrate, Rhodes grass as host and *G. fasciculatum* as VAM fungus, incorporation of captan and carbofuran in the substrate was also recommended to produce a high quality inoculum with maximum number of infective propagules (Bagyaraj 1990).

Sand-maize combination

In studies conducted at the Department of Agricultural Biochemistry, University of Hawaii, Honolulu, USA, *Glomus aggregatum* was cultured on maize grown on sand substrate. Fibreglass screen layers wrapped around the sand medium effectively provided a framework for sporocarp formation and facilitated the subsequent harvest of the sporocarps (Huang and Tang 1988).

In studies conducted at the Soil Microbial Systems Laboratory, United States Department of Agriculture, Agricultural Research Service, Beltsville, USA, two sets of experiments were conducted. In one set, pots were filled with either sand:soil:turface (1:1:1) (soil mix) or with sand only, having a particle size distribution of 4 850 mm, 80 280–850 mm, 16 280 mm. Potting media were inoculated throughout with either *Glomus etunicatum* B1 or *Gigaspora margarita* INVAM 105 and planted with corn cv. 'Iochief', soil mix was watered and fertilized (25-0-25) manually as needed for the 10-week study whereas sand-only pots were automatically drip-irrigated 3 times per day with 30 ml of a modified Hoagland's solution containing 10 mM N and 2 mM P at pH 6.1. The effect of 4-morpholine ethane sulfonic acid (MES) buffer on VAM fungal spore production was also examined. In another set of experiments, pots with sand of particle size distribution of 60 850 mm, 27 555–850 mm, and 13 550 mm were inoculated with either *G. etunicatum*, *G. mosseae* INVAM-156 or *Gigaspora margarita*, *Glomus mosseae*, pH was increased by the addition of 12 ml $CaCO_3$ per pot. The P concentration in irrigation solution was adjusted from 2 mM to 20 mM and was compared ultimately with 100 mM P. Cultures at pH 6.1 and 7.0 produced the same number of spores per gram as those grown on the soil mix even though the total root weight from sand cultures was significantly lower than in the soil mix. In subsequent experiments with higher-P-nutrient solutions, root weight increased significantly. The absence of soil organic matter and clay particles resulted in much cleaner spores from the sand culture system and the addition of MES buffer to control pH had no adverse effect on spore production. Time involved in preparation and handling was half that needed in soil mix pots. Advantage of spore production in sand also include culturing of VAM species with varying pH, ease of checking for contaminants, adaptability of sand media to storage, inoculum incorporation in test soils and ease in measurement of extraradical hyphal length density (Millner and Kitt 1990).

In further studies conducted at the above University, corn (*Zea mays*) plants were grown with *Glomus etunicatum*, *G. mosseae* or *Gigaspora margarita* in sand automatically irrigated with modified Hoagland's solution. Sand particle size, irrigation frequency, P concentration and buffer constituents were adjusted to maximize spore production. Modified half-strength Hoagland's solution, buffered with 4-morpholine ethane sulfonic acid (MES) automatically applied five times per day resulted in the production of 235 *G. etunicatum* spores per g dry weight of the medium (341 000 spores per pot) and 44 *G. margarita* spores per g dry weight of the medium (648 001 spores per pot). For six basophilic isolates of *G. mosseae*, $CaCO_3$ was incorporated into the sand and the pots were supplied with the same nutrient solution as for acidophilic isolates. The increased pH from 6.1 ± 0.2 to 7.2 ± 0.2 resulted in spore production from 70–145 spores per gram dry dry weight (102 000–210 000 spores per pot) (Millner and Kitt 1992).

B. On-farm production of VAM inoculum

Studies conducted at the Institute for Tropical and Subtropical Crop Science, Gottingen, Germany, showed that 3–5 t per ha inoculum (infected soil) was required to obtain inoculation response with cassava, beans or pasture plants. The on-farm production of such an amount of soil-based inoculum was therefore tested to minimize the cost of production and transportation. Dasomet was used as soil sterilant. A grass, *Brachiaria decumbens* was selected as the host and *Glomus manihotis* was selected since this is an effective fungal species in acid soils and produces 70 spores per g of soil after 4 months in the field. The estimated production cost for 5 t of soil inoculum was 5–6 dollars under Colombian conditions. A starter inoculum of *G. manihotis* is available in Colombia by the trade name MANIHOTINA (Sieverding 1987).

In studies at the Tata Energy Research Institute, New Delhi, India, a three-year experiment

was conducted for on-farm mass production of 7 exotic VAM isolates (*Glomus etunicatum*, *G. fasciculatum*, *G. intraradices*, 2 isolates, *G. mosseae* 2 isolates and *Gigaspora margarita*), one indigenous isolate of *G. caledonium* and a mixed indigenous cosortium of VAM species. Starter cultures were produced in pots and mass production was done on raised beds in a nursery site in three 4-month multiplication cycles each year for three years. The hosts used for three cycles were sudan grass (*Sorghum sudanese*), maize and carrot in first year, maize, sudan grass, and onion (*Allium cepa*) in the second year and sudan grass, maize, and oats (*Avena sativa*) in the third year. A 15–47 fold increase in infective propagules was obtained from first to third year of the experiment. Root colonization was greatest with the indigenous consortium which was 68% of the root length on carrots in the first year, 86% of the root length on onions in the second year and 87% of the root length on sorghum in the third year. In one isolate of *G. intraradices*, root colonization was 27% of the root length on sorghum in the first year and 68% of the root length on oats in the final year (Douds, Gadkar, and Adholeya 2000)

C. VAM inoculum production by aeroponic system

Production of VAM inoculum on roots produced by the aeroponic culture of host plants ensures freedom from plant and soil debris. Continuous harvesting of root inocula is possible with this system yielding a reliable source of biomass for immunological, biochemical and molecular genetic studies (Tester, Millner, and Kitt 1991). Also, the absence of the soil mineral component allows colonized root inocula to be sheared (finely cut). Sheared inocula can then be efficiently distributed by direct mixing with growth media or by incorporation in a flowable hydrogel suspension (Jarstfer and Sylvia 1992).

Studies conducted at the Department of Soil Science, University of Florida, Gainsville, Florida, USA, showed that in the aeroponic system, colonization and sporulation on the host plant by VAM fungi were superior to those reported for soil-based pot cultures. Uniform colonization (75%) of *Paspalum notatum* roots by *Glomus mosseae* and abundant sporulation (5 chlamydospores per cm colonized root length) were obtained after 8 weeks in aeroponic cultures. Colonization of roots by *Glomus intraradices* reached 50% after 12 weeks in an aeroponic culture and a mean of 8 chlamydospores per cm of colonized root were observed. This method should allow more efficient and uniform cultures of VAM (Sylvia and Hubbell 1986).

In further studies conducted at the above University, precolonized seedlings and a modified Hoagland's nutrient solution with a low phosphorus (1 mM) level were used. Three VAM fungal species (*Glomus deserticola, G. intraradices, G. mosseae*) were successfully established in aeroponic culture with bahia grass (*Paspalum notatum*) and industrial sweet potato (*Ipomoea batatas*). These fungi not only colonized the roots but also sporulated profusely. All colonized roots and spores of *G. mosseae* produced aeroponically were viable and could be used as effective inocula (Hung and Sylvia 1987).

Further studies conducted at the above University on *Paspalum notatum* and industrial sweet potato (*Ipomoea batatas*) inoculated with *Glomus deserticola*, *G. etunicatum* and *G. intraradices* and grown in aeroponic cultures showed colonization after 12–14 weeks, by the inoculated VAM fungi. Abundant vesicles and arbuscules were formed in the roots and profuse sporulation was detected within and outside roots. Sweet potatoes produced significantly more roots and spores per plant than *P. notatum* although the percentage of root colonized was similar in both hosts. The mean percent root colonization and sporulation per cm of colonized roots generally increased with time, although with some treatments, colonization declined by week 14. Spore production ranged from 4 spores per cm of colonized roots for *G. etunicatum* to 51 spores per cm of colonized root by *G. intraradices*. Infectivity trials with root inocula resulted in a mean of 38, 45, and 28% of *P. notatum* roots colonized by *G. deserticola*, *G. etunicatum*, and *G. intraradices*, respectively. The germination rates *G. etunicatum* spores produced in soil were significantly higher than those produced in aeroponic cultures (64% versus 46%) after incubation at 28°C in 2 weeks. However infectivity studies comparing *G. etunicatum* spores from soil and aeroponic cultures indicated no biological differences between the spore sources (Hung and Sylvia 1988).

Studies conducted at the Nalt Pingtung Polytech Institute, Department of Plant Ind, Pingtung, Taiwan, on the adzuki bean showed that spore production by aeroponic cultures was significantly higher than that by nutrient flow culture. Spore production in the culture of 0.5 strength Hoagland's solution containing ¼ amount of phosphorus was better than that of 0.5 strength Hoagland's solution containing the normal amount of phosphate or Taifei nutrient solution. Among the three species of VAM fungi used in the study, the spore production of *G. etunicatum* and *G. fasciculatum* was more abundant than that of *G. mosseae*. VAM fungal chlamydospores were produced on the surface of adzuki bean roots in erlenmeyer flasks, 60 days after incolulation with VAM fungi (Wang and Tschen 1994).

In studies conducted at the IPA, Sab Bcol Solo, AV Gel Sas Martin, 1371 Bongi, Caixa Postal 1022, Recife PE Brazil, VAM inoculum was produced using an aeroponic system consisting of a tank with 200 litres of nutrient solution pulverizing

the root plants through micro-irrigation nozzles. During the first 28 days of cultivation, the plants suffered an adaptation stress with a significant reduction of the root colonization rate and spore number. However, after this adaptation period, the AMF colonization rate increased significantly, stabilizing at 56 to 72 days in sweet potato plants inoculated with *Entrophospora colombiana* with 72 % of colonization. The number of spores recovered in the root system was 5082 and 156336 per g of root dry matter at 70 and 98 days, respectively. However, with *Gigaspora margarita* and *Glomus etunicatum*, the values were lower than expected (De Souza, Burity, Santo, et al. 1996).

In studies conducted at the University of Western Sydney, Macarthur, Department of Biology Science, Campbell Town, NSW, Australia, conventional atomizing disc aeroponic technology was compared with the latest ultrasonic nebulizer technology for the production of *Glomus intraradices* inocula. The piezo-ceramic element technology used in the ultrasonic nebulizer employs high frequency sound waves to nebulize nutrient solution into microdroplets, 1 micrometer in diameter. The growth of precolonized AM roots of sudan grass was achieved in both chambers used but both root growth and mycorrhization were significantly faster and more extensive in the ultrasonic nebulizer system than in the atomizing disc system. Shearing of the roots infected by AM fungi in both the systems did not reduce inoculum viability as was evident from the MPN data. However, sheared roots from the ultrasonic nebulizer system had significantly more infective propagules than those produced in the atomizing disc system. Thus the latest ultrasonic nebulizer aeroponic technology appears to be superior and an alternative to the conventional atomizing disc or spray nozzle system for the production of high quality AMF unocula. These inocula can be used in small doses to produce a large response which is a prerequisite for the commercialization of AMF technology (Mohammad, Khan, and Kuek 2000).

D. VAM inoculum production in hydroponic cultures

In studies conducted at the Institute fur Pflanzen Krankheiten und Pflanzenschutz der Universitat, Hannover, Hannover, Germany, VAM fungi were cultivated on various host plants in a hydroponic culture system. Inoculations were carried out with infected expanded clay from mycorrhizal stock cultures as particles of expanded clay were found to contain high level of spores and fungal mycelia. Inocula from stock cultures in sandy soils were also used for inoculation. Expanded clay was decontaminated with Pythium without substantial loss of mycorrhizal infectivity (Dehne and Baekhaus 1986).

In further studies conducted at the above Institute a hydroponic system was described for culturing and maintaining the VAM fungus, *Glomus intraradices* in symbiosis with linseed under glasshouse conditions in pure nutrient solution. With this system, it was possible to obtain large quantities of mycorrhizal host plant roots as well as extramatrical mycelium and chlamydospores free of impeding residues of solid substrate components. Starting from linseed donor plants inoculated in sand and transferred to the nutrient solution, new infections arose within the fast-growing root system, hyphae spread out into the liquid and infected mycorrhiza free receptor plants (Dugassa, Grunewaldt-Stocker, and Schonbeck 1995).

E. VAM inoculum production by the nutrient film technique

In the nutrient film technique, host plants, preinfected with VAM, are placed in an inclined tray over which flows a layer of nutrient solution. The pH of the nutrient solution can be adjusted to the requirement of individual isolates. For legumes, 0.05 to 0.10 strength Hoagland's solution with nitrogen can be used (Douds, Gadkar, and Adholeya 2000).

In studies conducted at the Microbiology Department, GB Pant University of Agriculture and Technology, Pant Nagar, Uttaranchal, India, four endophytes were multiplied on roots of NFT-grown mung plants and were successfully used as inoculum for maize (*Zea mays*) in pot cultures. *Gigaspora margarita* brought about 84% infection of roots in the NFT system after 40 days but *G. calospora* was not as effective (43%). *Glomus caledonium* and *G. fasciculatum* produced 68% and 50% infection of root in the NFT system. Root inculum produced in moong in the NFT system by *G. fasciculatum* was most effective when tested on maize while that produced by *G. calospora* was the least effective. Shoot dry weight in maize seedlings was higher when roots were infected with inocula from NFT-grown *G. fasciculatum* and *G. calospora* as compared to the other two endophytes. Root dry weight was however, greater with *G. margarita* as inoculum source (Mathew and Johari 1987, 1988).

F. In vitro cultivation of VAM inoculum

(i) On micropropagated plants

Studies conducted at the INRA Station d'Agronomic Dijon, Cedex, France, on dual axenic cultures of sheared-root inocula of VAM and tomato roots showed significant sporulation and the production of extensive biomass when surface-sterilized sheared-root inocula of *Glomus intraradices* and *G. versiforme* from pot cultures were associated with excised tomato roots. As many as $(10)^2 - 1(10)^3$ axenic mature spores were recovered in petridishes after three months incubation in the dark. Propagules of both species were able to complete their vegetative life cycle in vitro and efficiently colonize *Acacia albida* roots after one

month under glasshouse conditions. The effectiveness of 0.5 cm pieces of VAM roots as starter inocula indicates the high inculum potential of intravesicle propagules (Diop, Planchette, and Strullu 1994).

In studies conducted at the Department of Botany, JNU University, Jodhpur, Rajasthan, India, roots of in vitro grown plantlets of *Ziziphus nummularia* were inoculated with spores of *Glomus deserticola* and after 40 days maintenance in flasks under controlled conditions (60% humidity and 20°C–25°C temperature), the plantlets were transferred to sand in pots and maintained in a greenhouse. Spores of *G. deserticola* proliferated vigorously under in vitro conditions and produced external and internal hyphae, vesicles, and arbuscules (Mathur and Vyas 1995).

(ii) On Ri t-DNA transformed roots

Carrot roots transformed by the t-DNA of the Ri-plasmid of *Agrobacterium rhizogens* are an excellent vegetable host for the multiplication of VAM inoculum. Studies conducted at the Centre de Recherche en biologie Forestiere, Faculte de Foresterie et de Geomatique, Universite Laval, Quebec, Canada, on dual in vitro cultures of *Gigaspora margarita* and Ri t-DNA transformed roots of carrot showed that it was possible to calculate the percentage of mycorrhizal infection in an original manner based on the length of the infection units and the size of the whole root system. Correlations between this percentage and the other studied variables were highly positive. The establishment of VAM under aseptic conditions is simultaneously achieved by a multiplication and length enhancement of the infection units. Sporulation of *G. margarita* was noticed after six months of culture. Newly-produced mature spores were globular, white, and germinated readily in 4 germination tests on a solid medium (direct germination of newly-matured spores, with the presence of isolated roots occurring after surface sterilization and thermal shock) (Diop and Piche 1990).

In studies conducted at the University of Montreal, Institut de Recherche en Biologie Vegetate, Montreal, Quebec, Canada, *Glomus intraradices* was grown on genetically-transformed carrot *(Daucus carota)* roots in a two-compartment in vitro system. The growth of the mycorrhizal roots was restricted to one compartment (proximal) containing a complete growing medium. Only the endosymbiont was permitted to grow on to the second compartment (distal) containing the same medium lacking sugar. Colonization of the distal compartment by the mycelium took place between six and eight weeks after subculturing the mycorrhizal roots in a proximal compartment. Hyphal and spore densities were significantly higher in the distal compartment. Up to 34 000 spores with a mean of 15 000 mostly viable spores, per plate, were counted in the distal compartment (Arnaud St, Hamel, Vimarel, et al. 1996).

In further studies at the above University on a two-compartment aspetic petriplate system as described above, the medium in the distal compartment contained nitrogen either as NO_3^- or as NH_4^+. The pH and anion and cation concentration were measured every 15 days in filtrates prepared from the distal compartments. Basification (of the NO_3^- medium) and light acidification (NH_4^+ medium) occurred 13 weeks after colonization and no change in NO_3^- concentration. Treatments with N as NO_3^- showed no cation (Ca^{2+} and Mg^{2+} variation or anion (PO_4^{3-} and SO_4^{2-}) variation although the K^+ concentration significantly increased. Treatments with N as NH_4^+ showed no variation in cations or anions except for increases in the concentration of K^+ and Cl^- (Villegas, Williams, Nantais, et al. 1996).

Studies conducted at the Ecol Polytech. Dept. Chem. Engn. Bipro Res. Ctr. Montreal, Canada, a symbiotic culture of *Glomus intraradices* with *Daucus carota* hairy roots transformed by *Agrobacterium rhizogenes* showed that there was a critical inoculating condition at similar to 0.6 dry mass (DW) per litre medium. Above this critical condition growth was significantly reduced when using a low salt minimal (M) liquid medium, previously developed for hairy root-AM fungi co-cultures. Below critical inoculum conditions, the maximum specific root growth and specific *G. intraradices* spore production rates of 0.021 and 0.035 d^{-1}, respectively, were observed in the petri dish submerged culture system. Maximum spore production in the air lift bioreactor submerged culture system was ten times lower than that of petridish cultures and obtained with the lowest inoculum assessed (0.13 g DW per litre medium) with $1.82 \times 10^5 + 4.05 \times 10^4$ (SEM) spores per g DW inoculum per litre medium in 107 days. This work proposed a second generation bioprocess for AM fungi propagule production in bioreactors (Jolicoeur, Williams, Chaverie, et al. 1999).

In studies conducted at the Tata Energy Research Institute, New Delhi, India, on identification of a suitable host for mass production of VAM by Ri t-DNA technique, a number of clones of different plant species on the basis of their growth pattern were identified. These were ERRC-IA, ERRC IIA, GP IA, GP IIA, GP IIB for carrot; TOM-A for tomoto; Cu IA, Cu IIB, Cu IIIC for cucumber and CH-C for chilli. In compatibility studies with *Gigaspora margarita*, all the clones tested were positive in mycorrhizal symbiosis and there was not much variation in the time taken for incidence. Carrot clone GPIA was found to be the best as it was a fast-growing clone as well as compatible with the VAM fungus (Verma and Adholeya 1995).

Further studies conducted at the above Institute on dual in vitro cultures of *Gigaspora margarita* and Ri t-DNA transformed carrot roots showed that VAM fungi formed spores inside the host roots

in vitro. Sporulation was a temporal phenomenon found in dual cultures more than 18–20 months old. The spores were formed singly or rarely in clusters of 2–3. No preferential zone of formation was found. The spores formed intra-radically were 10%–15 % of the total spores formed in a single culture. The morphological studies and DNA polymorphism pattern of these intraradical spores and spores formed conventionally in the medium did not show any detectable variation (Gadkar and Adholeya 2000).

Studies conducted at the Universite d' Angers, Laboratoire de Phytonique, Cedex, France, on *Glomus versiforme* in a root segment inoculum associated with Ri t-DNA transformed carrot roots showed three phases of sporulation. These were a lag phase, a period of intensive production, and a plateau phase. An average of 9500 spores per petridish were produced after 5 months of dual culture. The root organ culture system supported extensive root colonization with the formation of many arbuscular and vesicles. The fungus both within root segments and as spores produced was viable and able to complete its life cyle in vitro. The mycorrhizal root segments, however, exhibited higher inoculum potential due to the numerous vesicles and extensive intraradical mycelium (Declerck, Strullu, and Plenchette 1996).

In studies conducted at the Univ. Catholique Lauvain, Unite Microbiol, Louvian, Belgium, five VAM fungi isolated from the rhizosphere of banana and sugarcane were successfully cultured in vitro, in association with genetically tranformed roots of carrot. The intraradical forms of fungi as mycorrhizal root pieces and single isolated vesicles constituted excellent sources of inoclum for the establishment of in vitro cultures and for the continuous culturing of the species. Several thousand fungal propagules were obtained for *G. versiforme* and *G. intraradices*, and to a lesser extent for *G. fasciculatum* whereas few spores were obtained for *G. macroearpum* (Declerck, Strullu, and Plenchette 1998).

References

Al-Raddad Ahmad M. 1995
Mass production of *Glomus mosseae* spores
Mycorrhiza **5**(3): 229–231

Arnaud St M, Hamel C, Vimard B, Caron M, and Fortin J A.1996
Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an in vitro system in the absence of host roots
Mycological Research **100**: 328–332

Bagyaraj D J. 1990
***Glomus fasciculatum* inoculum: its shelf life and the possibility of introducing rhizobium in it**
In *Innovation and Hierarchical Integration*, p.16, edited by M F Allen
Wyoming: University of Wyoming 324 pp.
[Proceedings of the Eighth North American conference on Mycorrhiza, 5–8 September 1990, Jackson, Wyoming]

Barbara A, Daniels J, and Menge A. 1979
Potential of *Glomus epigaeus* for commercial application
In *Proceedings of the 4th North American Conference on Mycorrhiza* edited by C P P Reid
Colorado: Colorado State University. 197 pp.
[Colorado, USA, 24–28 June 1979]

Declerck S, Strullu D G, and Plenchette C. 1996
In vitro mass-production of the arbuscular mycorrhizal fungus, *Glomus versiforme*, associated with Ri T-DNA transformed carrot roots
Mycological Research **100**(10): 1237–1242

Declerck S, Strullu D G, and Plenchette C. 1998
Monoxenic culture of the intraradical forms of Glomus sp. isolated from a tropical ecosystem: a proposed methodology for germplasm collection.
Mycologia **90** (4): 579–585

Dehne H W and Baekhaus G F. 1986
The use of vesicular-arbuscular mycorrhizal fungi in plant production. I. Inoculum production
Journal of Plant Diseases and Protection **93**(4): 415–424

De Souza E S, Burity H A, Santo A C D E, and daSilva M L R B. 1996
Alternative for arbuscular mycorrhizal fungi inoculum production in aeroponic culture
Pesquisa Agropecuaria Brasileira **31**(2): 153–158

Diop T A, Plenchette C, and Strullu D G. 1994
Dual axenic culture of sheared-root inocula of vesicular-arbuscular mycorrhizal fungi associated with tomato roots
Mycorrhiza **5**(1): 17–22

Diop T A and Piche Y. 1990
Long-term outcome of an endomycorrhizal symbiosis under aseptic conditions
In *Innovation and Hierarchical Integration*, p.81, edited by M F Allen
Wyoming: University of Wyoming 324 pp.
[Proceedings of the Eighth North American conference on Mycorrhiza, 5–8 September 1990, Jackson, Wyoming]

Douds D D, Gadkar V, and Adholeya A. 2000
Mass production of VAM fungus biofertilizer
In *Mycorrhizal Biology*, pp 197–215, edited by K G Mukerji and B P Chamola
New York: Kluwer Academic Publishers

Dugassa D G, Grunewaldt-Stocker G, and Schonbeck F. 1995
Growth of *Glomus intraradices* and its effect on linseed (*Linum usitatissimum* L.) in hydroponic culture
Mycorrhiza **5**(4): 279–282

Gadkar V and Adholeya A. 2000
Intraradical sporulation of AM *Gigaspora margarita* in long-term axenic cultivation in Ri T-DNA carrot root
Mycological Research **104**: 716–721

Hua S S T. 1990
Prospects for axenic growth and feasibility of genetic modification of vesicular-arbuscular mycorrhizal (VAM) fungi
In *Innovation and Hierarchical Integration*, p.145, edited by M F Allen
Wyoming: University of Wyoming 324 pp.
[Proceedings of the Eighth North American conference on Mycorrhiza, 5–8 September 1990, Jackson, Wyoming]

Huang R S and Tang C S. 1988
Production of vesicular-arbuscular mycorrhizal sporocarps *Glomus aggregatum* on fiberglass screens
Plant and Soil **108**(2): 233–35

Hung L L and Sylvia D M. 1987
VAM inoculum production by an aeroponic culture
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]

Hung L L and Sylvia D M. 1988
Production of vesicular-arbuscular mycorrhizal fungus inoculum in aeroponic culture
Applied Environmental Microbiology **54**(2): 353–57

Jarstfer A G and Sylvia D M. 1992
In *Proceedings of the International Symposium on Management of Mycorrhizas in Agriculture, Horticulture and Forestry*, p.99, edited by D Jasper
Australia: Australian Institute of Agricultural Sciences, 164 pp.
[28 September – 2 October 1992, University of Western Australia, Nedlands, Australia]

Jolicoeur M, Williams R D, Chavarie C, Fortin J A, and Archambault J. 1999
Production of *Glomus intraradices* propagules, an arbuscular mycorrhizal fungus, in an airlift bioreactor
Biotechnology and Bioengineering **63**(2): 224–232

Mathew J and Johri B N. 1987
Vesicular-arbuscular mycorrhizal inoculum through nutrient film technique (NFT)grown moong (*Phaseolus mungo*)
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]

Mathew J and Johari B N. 1988
Propagation of vesicular-arbuscular mycorrhizal fungi in moong (*Vigna radiata L*) through nutrient film technique (NFT)
Current Science **57**(3): 156–58

Mathur N and Vyas A. 1995
In vitro production of *Glomus deserticola* in association with *Ziziphus nummularia*
Plant Cell Reports **14**(11): 735–737

Mehrotra M D. 1996
Multiplication of VAMF on Paulownia - a veritable possibility
Indian Forester **122**(9): 858–860

Millner P D and Kitt D G. 1990
A soilless system for production of VAM fungus inoculum
In *Innovation and Hierarchical Integration*, p. 211, edited by M F Allen
Wyoming: University of Wyoming 324 pp.
[Proceedings of the Eighth North American conference on Mycorrhiza, 5–8 September 1990, Jackson, Wyoming]

Millner P D and Kitt D G. 1992
The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi
Mycorrhiza **2**: 9–15

Mohammad A, Khan A G, and Kuek C. 2000
Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi
Mycorrhiza **9**(6): 337–339

Sieverding E. 1987
On-farm production of VAM inoculum
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 S. 1999
Role of mycorrhiza in tree nurseries-Part III. Comparative performance of mycorrhizal fungi
Mycorrhiza News **11**(1): 2–9

Singh S. 2001
Mycorrhizal dependency, Part 1: selection of efficient mycorrhizal fungi
Mycorrhizal News **13**(1): 2–16

Sreenivasa M N and Bagyaraj D J. 1987
Selection of a suitable substrate for mass multiplication of *Glomus fasciculatum*
In Mycorrhiza Round Table: *Proceedings of a workshop,* pp. 592–599, 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]

Sreenivasa M N and Bagyaraj D J. 1988a
Optimum frequency of addition of nutrient solution for mass production of *Glomus fasciculatum* inoculum
Current Science **57**(12): 665–67

Sreenivasa M N and Bagyaraj D J. 1988b
Effect of culture, age and pruning on mass production of the VA mycorrhizal fungus

Glomus fasciculatum
Pertanika **11**(1): 143–45

Sreenivasa M N and Bagyaraj D J. 1989
Suitable form and level of phosphorus for mass production of the VA mycorrhizal fungus *Glomus faciculatum*
Zentralblatt fur Mikrobiologie **144**(1): 33–36

Sreenivasa M N and Bagyaraj D J. 1990
Suitable source and level of nitrogen for mass production of the VA mycorrhizal fungus *Glomus fasciculatum*
In *Current Trends in Mycorrhiza Research*, pp 35–36 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]

Sylvia D M and Burks J N. 1988
Selection of a vesicular-arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata*
Mycologia **80**(4): 565–68

Sylvia D M and Hubbell D H. 1986
Growth and sporulation of vesiclar arbuscular mycorrhizal fungi in aeroponic and membrane systems
Symbiosis **1**: 259–67

Talukdar N C and Germida J J. 1993
Propagation and storage of vesicular-arbuscular mycorrhizal fungi isolated from Saskatchewan agricultural soils
Canadian Journal of Botany - Revue Canadienne de Botanique **71**(10): 1328–1335

Tester C F, Millner P D, and Kitt D G. 1991
A misting apparatus for studying plant-microbe interactions and nutrient utilization
The Rhizosphere and Plant Growth **14**: 380

Verma A and Adholeya Alok. 1995
Selection of suitable host for in vitro inoculation by vesicular-arbuscular mycorrhizal fungi *Gigaspora margarita*
In *Mycorrhizae: Biofertilizers for the Future*, pp. 482–484, 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]

Villegas J, Williams R D, Nantais L, Archambault J, and Fortin J A. 1996
Effects of N source on pH and nutrient exchange of extramatrical mycelium in a mycorrhizal Ri T-DNA transformed root system
Mycorrhiza **6**(4): 247–251

Wang C L and Tschen J S M. 1994
Production of mycorrhizal inoculum by soilless culture
Journal of the Agricultural Association of China **167**: 50–60

Research findings

Variation among maize lines for colonization and responsiveness to arbuscular mycorrhizal fungi

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Introduction

It is generally accepted that AM (arbuscular mycorrhizal) fungi are non-specific in their selection of hosts (Gianinazzi-Pearson, Gianinazzi, and Trouvelot 1985). Compatibility between the host plant and their mycorrhizal fungi is a rule and incompatibility is an exception (Smith and Read 1997). Compatibility has been defined by Koide and Schreiner (1992) as the 'competence of both symbionts to engage a fully functioning symbiosis'. It is known that various degrees of interaction exist among compatible AM interactions. Furthermore, a particular AMF can infect a number of hosts but the extent of infection varies for a given soil fertility level. Phenotypic and genotypic variations in response to AMF colonization between plant cultivar or lines/genotypes of a single species, with respect to nutrient acquisition and growth (i.e. mycorrhizal dependency), have been reported (Azcon and Ocampo 1981; Krishna, Shetty, Dart, et al. 1985; Boyetchko and Tewari 1995; Rosewarne, Barker, and Smith 1997). However, differences in the relative mycorrhizal dependency between crop species or even cultivars are related to inherent factors such as root structure, metabolism, and plant growth rates which could affect nutrient demand (Koide 1991).

Substantial progress is being made towards understanding the interaction of plants and AM fungi by genetic and molecular analysis (Harrison 1997; Smith and Read 1997). However, the genetic basis of variation among genotypes and among species for the plant mycorrhizal interaction is not well understood, with little information available for maize.

The objective of this research was to characterize variation among a set of maize lines i.e. inbred,

hybrid, and composite for their relative ability to respond to AM symbiosis in a greenhouse.

Material and methods

Twenty six genetically diverse maize genotypes (inbred, composite, and hybrid lines) were analyzed (Table 1). Seeds were obtained from the Vivekanand Research Centre, Almora. Individual plants were grown in 500 ml earthen pots in a polyhouse maintained at 27°C (day temperature) and 24°C (night temperature) with a 16-h light and 8-h dark lighting regime. The soil for this experiment was a clay loam with a pH of 6.46 and 18 ppm available phosphorus. The soil was autoclaved before potting, with no obvious alteration in soil structure. For mycorrhizal treatments, a mycorrhizal inoculum consisting of spores, hyphae, and infected roots from plants grown in the same soil was mixed with autoclaved soil in ratio of 1:3 (inoculum: autoclaved soil mixture). Uninoculated plants were given the filtrates of inoculum soil to ensure that they received the same consortium of microorganisms as the mycorrhizal plants. Three replicates were maintained for each line.

Plants were harvested after 40 days of emergence. Dry weight was measured on the total above-ground portion of the plant. The roots were cleared and stained using a modification of the technique described by Phillips and Hayman (1970), in which lactoglycerine was substituted for lactophenol. The per cent mycorrhizal/arbuscular colonization of the root was determined by the method of Biermann and Linderman (1981).

Mycorrhizal colonization values were angularly transformed.

Table 1 Mean shoot weight of 26 maize lines grown in clay loam soil with or without the addition of arbuscular mycorrhizal fungi

Lines	*Shoot weight (g)*		*Response to mycorrhizae (%)*[#]	*Mycorrhizal colonization (%)*	*Arbuscular colonization (%)*
	*M+**	*M–**			
Inbred					
CM 126	0.40	0.22	64.86	45.19	44.25
CM 129	0.26	0.24	21.17	22.40	5.50
Hybrid					
FH 3044	0.72	0.51	39.92	31.67	15.00
FH 3054	0.94	0.54	59.42	45.05	30.0
FH 3048	0.84	0.48	60.06	45.30	24.00
FH 3074	0.72	0.41	60.43	35.15	30.00
FH 3077	1.29	0.74	59.56	45.28	20.00
FH 3079	0.85	0.58	43.01	37.16	22.00
FH 3084	1.12	0.64	60.05	45.17	16.50
FH 3085	0.20	0.11	64.91	44.99	30.00
FH 3086	0.88	0.63	39.98	44.65	16.00
FH 3087	0.89	0.66	36.18	42.20	6.50
FH 3088	0.69	0.47	43.17	42.99	15.80
FH 3094	0.68	0.51	35.27	32.90	12.40
FH 3097	0.49	0.38	32.55	31.55	16.50
FH 3098	0.70	0.46	46.27	39.42	24.26
HIM 129	0.70	0.46	46.26	45.19	35.00
Vivek Hybrid 4	0.91	0.68	35.57	40.06	15.14
Composite					
VL makka 16	0.68	0.50	38.10	28.40	12.50
VL makka 88	0.91	0.71	38.33	34.13	6.06
VL makka 41	1.20	0.99	25.24	23.99	1.00
VL 78	0.50	0.36	38.58	29.51	17.50
VL 89	1.00	0.55	60.29	45.13	21.28
VL 90	0.98	0.63	39.23	33.35	24.10
Single cross parents					
CM 126* CM 127	0.96	0.55	59.72	45.32	21.00
CM 128* CM 129	0.45	0.35	32.31	33.66	15.26
CD at 5%	0.04		2.20	1.01	2.35

*M^+ = Mycorrhizal

*M^- = Non-mycorrhizal

#$[(M^+ - M^-)/M^- * 100]$ (Mycorrhizal reponsiveness = {[Shoot dry weight of mycorrhizal plant(M+)]–[Shoot dry weight of non-mycorrhizal plant(M^-)]/[Shoot dry weight of non-mycorrhizal plant] * 100}

Results and discussions

There was considerable variability among the maize lines with respect to mycorrhizal colonization; the range varied from 22.4% (CM129) to 45.32% (CM 126⋆CM127) for the twenty six lines. The highest mycorrhizal colonization was recorded in CM 126, FH 3054, FH 3048, FH 3077, FH 3084, FH 3085, FH 3086, HIM 129, VL 89, and CM 126*CM 127. Significant variation within composite and hybrid lines was also observed. However, composites in general had a lower level of infection than the hybrids (Table 1). Root colonization by mycorrhizal fungi varied considerably depending on the genotype, which might also be related to specific interactions between mycorrhizal fungi and the host species.

Although a proper explanation for such colonization is still unknown, it could be due to differential control of infection by plant genotype which would be mediated via the carbohydrate supply in conjunction with modifications in the membrane transport processes. It could also be a result of the presence or absence of the genes which are responsible for the establishment of a perfectly compatible symbiosis, since genotypes differ in their genetic makeup (Smith and Smith 1990). This indicates that plant symbionts exert a degree of control over fungal development (Gianinazzi-Pearson 1996).

Menge (1983) believes that rapid and high levels of colonization may be the prime determinant of efficiency of the symbiosis. Therefore, genotypes that prevent or allow lower levels of colonization are destined to derive meagre benefits from AM symbiosis. Contrary to these reports, the correlation between mycorrhizal responsiveness

based on shoot dry weight and colonization was non-significant in the present investigation. Genotype CM 126*CM 127 with 45.32% colonization gave 59.72% mycorrhizal responsiveness, at the same time genotype Vivek hybrid 4, inspite of having 45.94% mycorrhizal colonization could respond only 46.26% to mycorrhizal inoculation. Genotype FH 3074 showed 60.43% mycorrhizal responsiveness inspite of having only 35.15% of the root infected.

An important issue in interpreting the results of this study is the proportion of genetic variation responsible for mycorrhizal responsiveness that can be attributed to differences in plant-microbe interaction versus the proportion that can be attributed to phosphorus requirement of a cultivar at a given soil phosphorus level i.e. the degree to which a genotype can respond. A low correlation between arbuscular frequency and mycorrhizal responsiveness was observed in this study. Genotypes FH 3084 and FH 3077 are unique lines in that they showed a relatively higher mycorrhizal responsiveness despite having a lower degree of arbuscular colonization. These genotypes are a stark representation of different interpretations that can be made on the basis of different measures of plant-mycorrhizal interactions.

Our results support the previous conclusion that colonization (mycorrhizal/arbuscular) is not a good indicator of arbuscular mycorrhizal efficiency for plant growth (Schubert and Hayman 1986; Vierheilig and Ocampo 1989). The external phase of infection also needs to be considered for better understanding of the impact of these fungi. Graham, Linderman, and Menge (1982) had suggested that the amount of external mycelium was indicative of the efficiency of an arbuscular mycorrhizal infection.

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References

Azcon R and Ocampo J A. 1981
Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars
The New Phytologist **87**: 677–685

Biermann B and Linderman R G. 1981
Quantifying vesicular-arbuscular mycorrhizae. A proposed method towards standardization
The New Phytologist **87**: 63–69

Boyetchko S M and Tewari J P. 1995
Susceptibility of barley species to vesicular-arbuscular mycorrhizal fungi
Canadian Journal of Plant Science **75**: 269–275

Gianinazzi-Pearson V. 1996
Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis
Plant Cell **8**: 1871–1883

Gianinazzi-Pearson V, Gianinazzi S, and Trouvelot A. 1985
Evaluation of infectivity and effectiveness of indigenous vesicular arbuscular fungal population in some agricultural soil in Burgundy
Canadian Journal of Botany **63**: 1521–1524

Graham J H, Linderman R G, and Menge J A. 1982
Development of external hyphae by different isolates of mycorrhizal Glomus spp. in relation to colonization and growth of troyer citrange
The New Phytologist **91**: 183–189

Harrison M. 1997
The arbuscular mycorrhizal symbiosis: an underground association
Trends in Plant Sciences **2**: 54–60

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

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

Krishna K, Shetty K G, Dart J, and Andrew D J. 1985
Genotype dependent variation in mycorrhizal colonization and response to inoculation of pearl millet
Plant Soil **86**: 113–125

Menge J A. 1983
Utilization of vesicular-arbuscular mycorrhizal fungi in agriculture
Canadian Journal of Botany **61**: 1015–1024

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

Rosewarne G M, Barker S J, and Smith S E. 1997
Production of near-synchronous fungal colonization in tomato for development and molecular analyses of mycorrhiza
Mycological Research **101**: 966–970

Schubert A and Hayman D S. 1986
Plant growth responses to vesicular-arbuscular mycorrhiza. XVI. Effectiveness of different endophytes at different levels of soil phosphate
The New Phytologist **103**: 70–90

Smith S E and Read D J. 1997
***Mycorrhizal symbosis*, 2nd edition**
Cambridge, UK: Academic Press

Smith S E and Smith F A. 1990
Structure and function of the interfaces in biotrophic symbiosis as they relate to nutrient transport
The New Phytologist **114**: 1–38

Vierheilig H and Ocampo J A. 1989
Relationship between SDH-activity and VA mycorrhizal infection
Agriculture Ecosystem Environment **29**: 439–442

A new species of *Glomus* from India

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Introduction

AM (arbuscular mycorrhizal) fungi are widely distributed in non-fertile and nutrient deficient semi-arid tropical soils. During a survey of arbuscular mycorrhizal fungi associated with the rhizospheric soils of *Tylophora indica* Burns F, the authors collected a new species of *Glomus* following the wet sieving and decanting method (Gerdemann and Nicholson, 1963).

The present isolate has some similarities with *Glomus constrictum* Trappe. However the present species differs from the above in having a lateral bulbous swelling or knob-like structure located near the base of the spore and in the attachment of subtending hyphae. The chlamdospores of the present isolate are smaller in size than *Glomus constrictum* (Schenck and Perez 1987; Trappe, 1977). There is no fungus comparable with the present isolate, and it is hence described as a new species, *Glomus indica* sp. nov.

Chlamydospores naked, form singly in soils sub-globose to globose, 117 x 129 mm, dark brown, shiny-smooth, spore walls 5–10 mm thick, one-layered or occasionally two-layered, base straight or occasionally with a short funnel-shaped projection, followed by subtending hypha, constricted at the base, on one of the lateral sides at the base, a knob-like protuberance is present, attached hyphae straight to recurved and with the following features appearing in sequence away from the spore. At the point of attachment dark brown walls 3–6 mm thick, just beyond the point of attachment the hyphae constricts to 10 mm dia, just beyond the constriction, the hypha inflated to 15-30 mm dia with yellow to yellow-brown walls, 2–3 mm thick.

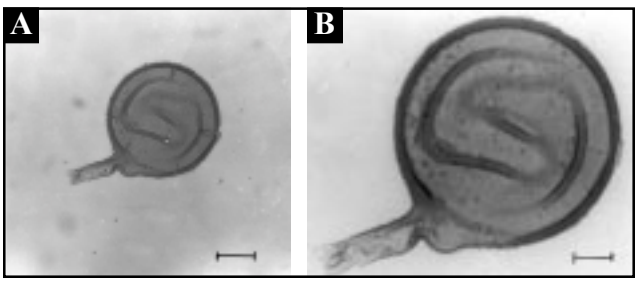

Figure 1 *Glomus indica* sp. nov. – spore with knob like structure near the hyphal attachment (A: scale bar = 50 µm, 400X; B: scale bar = 25 µm, 200X)

Distribution and mycorrhizal association

Botanical garden, Osmania University, Hyderabad, India, rhizosphere soil of *Tylophora indica*. Burm. F. HCIO-43864, OUFH-144, Date: 06/01/2001.

Chlamydospores singulatum, subglobosae vel globosae 117 × 129 mm, fuscae vel nigrae; paraetibus fussis 5-10 crasis, basibus rectis infundibuliformis, vel bulbosa lateraliis ad 5 × 7.5 mm. Hypha affixa recta vel recurvata ad basis spores 20–30 mm diametro, prope sporae typica constrica ad 10–20 mm diametro, sub constrictio typica tumida ad 15–30 mm at hyphis tenuibus protrudentibus.

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References

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

Schenck N C and Perez Y. 1990
Manual for the identification of VA mycorrhizal fungi
Gainesville, Florida: Synergistic Publications

Trappe J M. 1977
Three new endogonaceae: *Glomus constrictus*, *Sclerocystis clavispora* and *Acaulospora scrobiculata*
Mycotaxon **6**:359–368

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A simple technique for clearing adherent debris of VAM fungal spores for identification

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For the isolation and identification of VAM fungal spores from soil, the most commonly used technique is that of wet sieving and decanting adopted by Gerdemann and Nicholson (1963). The spores thus extracted frequently have roughened outer walls often with portions of sloughed wall fragments and adhering organic soil debris. The soil debris can create problems during identification, making it difficult to distinguish between the clear outer and inner wall layers. This problem was move pronounced when we were trying to isolate VAM fungal spores from fly ash-polluted soils collected from the ash bund area of the Raichur Thermal Power Station, Shakti Nagar, Raichur District, Karnakata. We found was that it was impossible to distinguish the spores from ash particulates in sieved washings, because the ash particles in the sieves also looked like haloes around bodies similar to spores (Figure 1A). Upon breaking them under the coverslip on a slide, even broken fragments looked more like broken spore walls (Figure 1B). However, with great difficulty one could 'suspect' VAM spores whose (Figure 1C) features were completely concealed under the ash coating making identification difficult. Even repeated washing of these spores with water (unlike with spores extracted from ordinary soils), could not help to clear the adherent ash debris which appeared more like a cemented matrix. Separation of VAM spores from organic spoil debris by differential sedimentation on a gelatin column as suggested by Mosse and Jones (1968) also failed to yield good results when used for the separation of VAM spores from fly ash particulates.

In order to overcome this problem, a method originally used for maceration of coal sedimentary matrix (Moore, Web, and Collinson 1991) to separate microfossils and spores was suitably modified and used.

This technique involves washing the slurry of soil (rhizospheric soil samples collected from ash bund area, where common weeds were growing was used) through a graded series of soil sieves (100, 240, and 400 BSS meshes). The residual suspension containing the ash-bound spores (Figure 1A) was first decanted into a graduated beaker and treated with 5% HCl (concentration of acid is adjusted depending upon the quantity of suspension) for 5 minutes followed by dilution with water before passing through the stack of sieves again. The residue was decanted into a beaker again through water washings. This was further treated with hydrofluoric acid for 30 minutes, and again diluted with water and washed through the set of sieves as earlier. The material retained on the sieves was then decanted into petriplates and the spores were observed under a stereomicroscope. The spores thus extracted and isolated were mounted on clear slides using either lactophenol or PVLG (polyvinyl alchol+ lactic acid+glycerol). It was only after the treatment with acid that the VAM spores could be seen. They were completely free from any adherent ash debris (Figure 1D) and all minute details were clear including wall layers, as seen in Figures 1E and 1F.

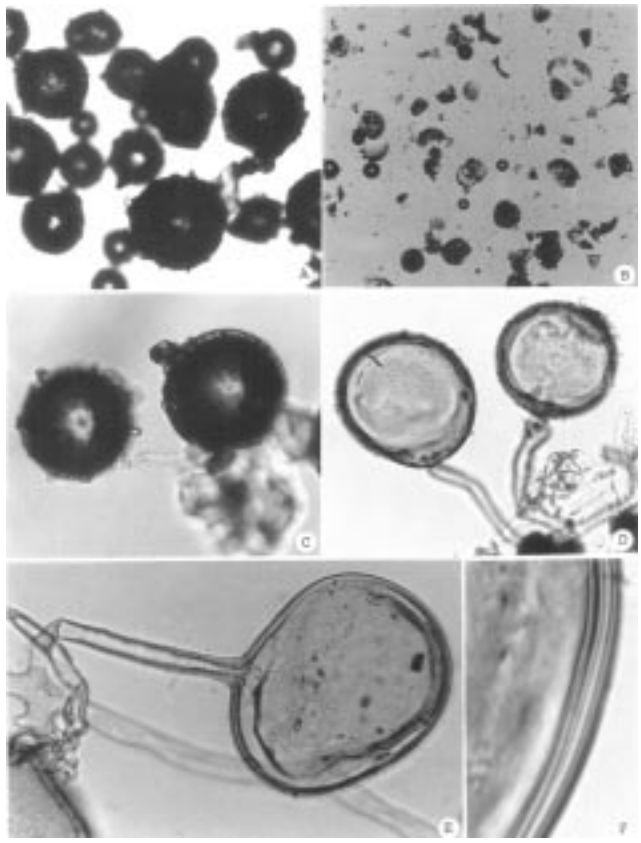

Figure 1 (A) A mixture of fly ash particulates and indistinguishable VAM spores decanted from sieves (× 20); (B) Fly ash particulate fragments after crushing, looking more like spore wall fragments (× 10); (C) Ash-covered chlamydospores after repeated washing (× 20); (D) Ash-cleared chlamydospores of Glomus isolated after maceration treatment (× 20); (E) Glomus spores showing clear characteristic features after treatment (× 40); (F) Spore wall showing distinct layers after treatment (× 100)

This technique was found to be useful in clearing VAM fungal spores from associated soil debris particularly from material such as fly ash.

References

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

Mosse B and Jones G W. 1968
Separation of *Endogone* species from organic soil debris by differential sedimentation on gelatin columns
Transactions of the British Mycological Society **51**: 604–608

Moore P D, Webb T A, and Collinson M E. 1991
Pollen Analysis, p. 43, 2nd edition
London: Blackwell Scientific Publications

Arbuscular mycorrhizal root colonization of some pulses in 7 districts of eastern Uttar Pradesh–a preliminary report

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Introduction

Pulses are important food crops in India. These are normally grown on marginal lands. The N requirement of such crops is met through symbiotic rhizobia, but their P and moisture requirements are fulfilled, partially by mycorrhizal fungi (Barea, Escudera, and Aquilar 1980; Schenck and Hinson 1973). Mycorrhizal association in plants is the rule rather than the exception (Gerdemann 1968). The absorption of minerals and water is influenced by the degree of mycorrhization (Harley and Smith 1983). Since pulses are grown under nutrient and moisture deficient conditions, an attempt was made to examine the degree of mycorrhization of some important pulses grown on the marginal lands of seven districts of eastern Uttar Pradesh.

Materials and methods

Surveys were carried out during the vegetative crop growing period. Root and soil samples were collected from fields of chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), mungbean (*Phaseolus*

Table 1 Mycorrhizal colonization of some pulses in seven districts of eastern Uttar Pradesh

Crop	*Mycorrhizal colonization (%)*	*Bahraich*	*Faizabad*	*Gonda*	*Jaunpur*	*Mirzapur*	*Sultanpur*	*Varanasi*
Chickpea	Range	62-90	30-90	30-85	30-88	45-92	20-90	35-90
	Mean± SD	78.1± 7.5	58.4±18.5	55.9±16.4	62.6±18.1	68.3±15.3	47.5±19.1	66.1±14.0
	n	25	35	15	20	20	20	25
Lentil	Range	68-90	30-82	30-70	33-82	48-82	22-70	52-85
	Mean± SD	78.9±6.7	56.5±15.5	51.8±17.2	54.1±16.0	66.2±11.7	44.6±14.4	68.7±11.1
	n	10	15	5	10	10	10	10
Mungbean	Range	62-88	40-77	25-82	40-90	48-90	32-82	35-90
	Mean± SD	74.3±8.1	60.6±11.8	60.4±18.0	68.4±15.8	68.5±13.1	52.2±14.7	66.8±16.7
	n	10	10	10	10	15	20	25
Peas	Range	50-89	30-95	40-88	35-90	53-80	20-85	38-85
	Mean± SD	74.5±11.6	64.0±17.7	63.5±13.3	63.9±18.6	70.3±9.3	50.6±19.2	65.7±16.3
	n	15	25	15	20	10	25	20
Pigeonpea	Range	45-88	30-90	35-90	32-88	30-82	18-90	40-98
	Mean± SD	65.3±13.0	60.1±19.6	66.1±17.5	60.3±16.5	57.5±15.9	48.8±19.3	63.3±14.8
	n	20	30	20	25	25	35	35
Urdbean	Range	50-80	32-80	32-88	30-85	30-81	18-82	35-90
	Mean± SD	67.1±10.5	58.3±17.8	60.3±16.6	65.3±16.1	59.1±15.9	45.6±17.8	66.4±16.2
	n	10	15	15	15	15	20	20

n – Number of root samples; SD – Standard deviation

aureus Roxb.), peas (*Pisum sativum* L.), pigeonpea (*Cajanus cajan* (L.) Mill sp.) and urd bean (*Phaseolus mungo* Roxb.) in a polythene bag, tagged, sealed with a rubber band and brought to the laboratory. Roots were properly washed under running tap water and cleaned in near-boiling 10% KOH aqueous solution for 48 hours. Such roots were stained in trypan blue following several washings in distilled water to drain out KOH (Phillips and Hayman, 1970). Stained roots were cut into 1-cm segments and 100 such segments were randomly picked up and examined under a stereoscopic microscope. Arbuscular mycorrhizal colonization was determined by Nicholson's formula (1955) as follows:

$$\text{Per cent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of segments observed}} \times 100$$

Results and discussions

It is evident from Table 1 that all the six pulses were colonized by arbuscular mycorrhizae to varying degrees (18% to 98%) in the seven districts of eastern Uttar Pradesh surveyed. The average percentage of colonization of these crops was lower (44.6% to 52.2%) in Sultanpur than in the remaining five districts (51.8% to 78.9%). This might be because a vast stretch of the land is salt-affected (pH 8–10.5) and has been brought under cultivation. Reports indicate that mycorrhizal fungi tend to adapt themselves to saline conditions (Azcon, Barea, and Hayman, 1976; Hirel and Gerdemann 1980), however, it is difficult to correlate the soil pH with mycorrhization (Frey and Ellis 1970). The average percentage of mycorrhization in the pulses of Bahraich district was higher (65.3% to 78.9%) compared to the other districts. This might be due to the sandy nature of soil of the localities from where samples were collected. Gianinazzi-Pearson, Gianinazzi, and Trouvelot (1985) evaluated the infectivity and effectiveness of indigenous arbuscular mycorrhizal fungal populations in sandy, clay, loam, and sandy-clay soils and found that it varied with soil type but was not necessarily related to the soil's physiochemical properties.

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References

Azcon R, Barea J M, and Hayman D S. 1976
Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate solubilizing bacteria
Soil Biology and Biochemistry **13**: 19–22

Barea J M, Escudera J L, and Aquilar A G. 1980
Effect of introduced and indigenous VA-mycorrhizal fungi on nodulation, growth and nutrition of *Medicago sativa* in phosphate fixing soils as affected by P fertilizers
Plant and Soil **54**: 283–285

Frey J E and Ellis J R. 1997
Relationship of soil properties and soil amendments to response of *Glomus intraradices* and soybeans
Canadian Journal of Botany **75**: 483–491

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

Gianinazzi-Pearson V, Gianinazzi S, and Trouvelot A. 1985
Evaluation of the infectivity and effectiveness of indigenous vesicular-arbuscular fungal populations in some agricultural soils in Burgundy
Canadian Journal of Botany **63**: 1521–1524

Harley J L and Smith S E. 1983
Mycorrhizal symbiosis
London: London Academic Press

Hirel M C and Gerdemann J W. 1980
Improved growth of onion and bell pepper in saline soils by two vesicular arbuscular mycorrhizal fungi
Soil Science Society of America Journal **44**: 1413–1425

Nicholson T H. 1955
The Mycotrophic Habit in Grass
Thesis paper, University of Nottingham, Nottingham, UK

Phillips J M and Hayman D S. 1970
Improved proceduring for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection
Transactions of the British Mycological Society **54**: 53–63

Schenck N C and Hinson K. 1973
Response of nodulatimg and non-nodulating soybeans to a species of *Endogone* mycorrhiza
Agronomy Journal **65**: 849–858

New approaches

A simple and rapid DNA extraction protocol for PCR in mycorrhizal fungi

A simple and rapid DNA extraction protocol for PCR in mycorrhizal fungi was developed by Manian S, Sreenivas Prasad S, Mills PR, which requires only minute quantities of starting material and which is

suitable for mycorrhizal fungi as well as a range of other fungi (Letters in Applied Microbiology 33(4): 307–310, 2001). The protocol combines the application of rapid freezing and boiling cycles and passage of the extracts through DNA-purification columns. PCR-amplifiable DNA was obtained from a number of endo- and ecto-mycorrhizal fungi using minute quantities of spores and mycelium, respectively. DNA extracted following this method was used to successfully amplify regions of interest from high as well as low copy number genes. The amplicons were suitable for downstream applications such as sequencing and PCR-RFLPs. The protocol described is simple, short and facilitates rapid isolation of PCR amplifiable genomic DNA from a large number of fungal isolates in a single day.

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.

- *Arid Land Research and Management*
- *Austral Ecology*
- *Australian Journal of Agricultural Research*
- *Brazilian Journal of Microbiology*
- *Cryptogamie Mycologie*
- *Diversity and interaction in a temperate forest community*
- *Ecological Monographs*
- *Ecological Research*
- *Ecology Letters*
- *FEMS Microbiology Ecology*
- *Fungal Diversity*
- *Journal of Experimental Botany*
- *Microbiological Research*
- *Mycorrhiza*
- *New Forests*
- *New Zealand Journal of Botany*
- *Pedobiologia*
- *Plant Molecular Biology*
- *Restoration Ecology*
- *Silva Fennica*
- *Techniques in Mycorrhizal Studies*
- *Trends in European Forest Tree Physiology Research*
- *Vitis*
- *World Journal of Microbiology & Biotechnology*
- *Zeitschrift Fur Pflanzenkrankheiten and Pflanzenschutz - Journal of Plant Diseases and Protection*

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]
He X L, Mouratov S, and Steinberger Y*. 2002	**Spatial distribution and colonization of arbuscular mycorrhizal fungi under the canopies of desert halophytes** *Arid Land Research and Management* **16**(2): 149–160 [*Steinberger Y, Bar Ilan University, Faculty of Life Science, IL-52900 Ramat Gan, Israel]
Packham J M, May T W*, Brown M J, Wardlaw T J, and Mills A K. 2002	**Macrofungal diversity and community ecology in mature and regrowth wet eucalyptus forest in Tasmania: A multivariate study** *Austral Ecology* **27**(2): 149–161 [*May TW, Royal Botanical Gardens Melbourne, Birdwood Ave, S Yarra, Victoria 3141, Australia]
Osborne L D*, and Rengel Z. 2002	**Genotypic differences in wheat for uptake and utilisation of P from iron phosphate** *Australian Journal of Agricultural Research* **53**(7): 837–844 [*Osborne L D, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia]
Carrenho R*, Silva ES, Trufem S F B, and Bononi V L R. 2001	**Successive cultivation of maize and agricultural practices on root colonization, number of spores and species of arbuscular mycorrhizal fungi** *Brazilian Journal of Microbiology* **32**(4): 262–270 [*Carrenho R, Univ Estadual Maringa, Department of Biology, Av Colombo 5790,BR-87020900 Maringa, Parana, Brazil]

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]
Bressan W*. 2001	**The interactive effect of phosphorus and nitrogen on ''in vitro'' spore germination of *Glomus etunicatum* Becker & Gerdemann, root growth and mycorrhizal colonization** *Brazilian Journal of Microbiology* **32**(4): 276–280 [*Bressan W, Embrapa Milho and Sorgo, Department of Microbiology, BR-35701970 Sete Lagoas, MG, Brazil]
NezzarHocine H* Abdesselam M, Guinberteau J, HalliHargas R, Saidi F, and Chevalier G. 2002	**Fungal macroflora of *Cedrus atlantica*. III - Relations between climate and basidiocarpogenesis** *Cryptogamie Mycologie* **23**(1): 19–37 [*Nezzar-Hocine H, University Mouloud Mammeri, Faculty of Science, Unite Recherche Biologie and Agroforesterie, Route Hasnaoua, Tizi Ouzou 15000, Algeria]
Okabe H*. 2002	**Dynamics of ectomycorrhizas and actinorhizal associations** *Diversity and interaction in a temperate forest community* **158**: 273–284 [*Okabe H, Forestry and Forest Production Research Institute, POB 16, Tsukuba, Ibaraki 3058687, Japan]
Pregitzer K S*, DeForest J L, Burton A J, Allen M F, Ruess R W, and Hendrick R L. 2002	**Fine root architecture of nine North American trees** *Ecological Monographs* **72**(2): 293–309 [*Pregitzer KS, Michigan Technology University, School of Forestry and Wood Production, Houghton, MI 49931 USA]
Setala H*. 2002	**Sensitivity of ecosystem functioning to changes in trophic structure, functional group composition and species diversity in belowground food webs** *Ecological Research* **17**(2): 207–215 [*Setala H, University of Helsinki, Department of Ecology and Environmental Science, Niemenkatu 73, FIN-15140 Lahti, Finland]
Gange A C* and Brown V K. 2002	**Soil food web components affect plant community structure during early succession** *Ecological Research* **17**(2): 217–227 [*Gange AC, University of London, Royal Holloway and Bedford New College, School of Biology Science, Egham TW20 0EX, Surrey, England]
Gange A C*, Stagg P G, and Ward L K. 2002	**Arbuscular mycorrhizal fungi affect phytophagous insect specialism** *Ecology Letters* **5**(1): 11–15 [*Gange A C, Royal, Holloway, University of London, School of Biological Science, Egham TW20 0EX, Surrey, England]
Founoune H, Duponnois R*, Meyer J M, Thioulouse J, Masse D, Chotte J L, and Neyra M. 2002	**Interactions between ectomycorrhizal symbiosis and fluorescent pseudomonads on Acacia holosericea: isolation of MHB (mycorrhiza helper bacteria) from a Soudano-Sahelian soil** *FEMS Microbiology Ecology* **41**(1): 37–46 [*Duponnois R, UR IBIS Interact BIol Sols Syst Anthropise Tropic, IRD, BP 182, 01, Ouagadougou, BURKINA FASO
Cafaro M J*. 2002	**Species richness patterns in symbiotic gut fungi (Trichomycetes)** *Fungal Diversity* **9**: 47–56 [*Cafaro MJ, University of Kansas, Department of Ecology and Evolutionary Biology, Lawrence, KS 66045 USA]
GarciaGarrido J M*, and Ocampo J A. 2002	**Regulation of the plant defence response in arbuscular mycorrhizal symbiosis** *Journal of Experimental Botany* **53**(373): 1377–1386 [*Garcia-Garrido J M, CSIC, Estac Expt Zaidin, Department of Microbiology Suelo and Sistemas Simbiot, Prof Albareda 1, E-18008 Granada, Spain]
Laatikainen T* and HeinonenTanski H. 2002	**Mycorrhizal growth in pure cultures in the presence of pesticides** *Microbiological Research* **157**(2): 127–137 [*Laatikainen T, University of Kuopio, Department of Environmental Science, POB 1627, FIN-70211 Kuopio, Finland]
Gaspar M L *, Cabello M N, Cazau M C, and Pollero R J. 2002	**Effect of phenanthrene and *Rhodotorula glutinis* on arbuscular mycorrhizal fungus colonization of maize roots** *Mycorrhiza* **12**(2): 55–59 [*Instituto de Investigaciones Bioquímicas de La Plata, 60 y 120, 1900 La Plata. Argentina]

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]
Becerra A*, Daniele G, Domínguez L, Nouhra E, and Horton T. 20002	**Ectomycorrhizae between *Alnus acuminata* H.B.K. and *Naucoria escharoides* (Fr.:Fr.) Kummer from Argentina** *Mycorrhiza* **12**(2): 61–66 [*Instituto Multidisciplinario de Biología Vegetal (CONICET). C C 495, 5000 Córdoba, Argentina]
Maldonado-Mendoza I E,* Dewbre G R, van Buuren M L, Versaw W K, and Harrison M J. 2002	**Methods to estimate the proportion of plant and fungal RNA in an arbuscular mycorrhiza** *Mycorrhiza* **12**(2): 67–74 [*The Samuel Roberts Noble Foundation, Plant Biology Division, 2510 Sam Noble Parkway, Ardmore, OK 73402, USA]
Young B W*, Massicotte H B, Tackaberry L E, Baldwin Q F, and Egger K N. 2002	***Monotropa uniflora*: morphological and molecular assessment of mycorrhizae retrieved from sites in the Sub-Boreal Spruce biogeoclimatic zone in central British Columbia** *Mycorrhiza* **12**(2): 75–82 [*Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, Canada N9B 3P4]
Wu B*, Nara K, and Hogetsu T. 2002	**Spatiotemporal transfer of carbon-14-labelled photosynthate from ectomycorrhizal *Pinus densiflora* seedlings to extraradical mycelia** *Mycorrhiza* **12**(2): 83–88 [*Research Unit for Symbiotic Function, Asian Natural Environmental Science Center, The University of Tokyo, Midori-cho 1-1-8, Nishitokyo, Tokyo 188-0002, Japan]
Urcelay C. 2002	**Co-occurrence of three fungal root symbionts in *Gaultheria poeppiggi* DC. in Central Argentina** *Mycorrhiza* **12**(2): 89–92 [*Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-Universidad Nacional de Córdoba, C.C. 495, 5000 Córdoba, Argentina]
Wang B, Funakoshi D M, Dalpé Y, and Hamel C. 2002	**Phosphorus-32 absorption and translocation to host plants by arbuscular mycorrhizal fungi at low root-zone temperature** *Mycorrhiza* **12**(2): 93–96 [*Department of Natural Resource Sciences, Macdonald Campus of McGill University, 2111 Lakeshore Road, Ste-Anne-de-Bellevue, Quebec, H9X 3V9 Canada]
Morandi D, Gollotte A, and Camporota P. 2002	**Influence of an arbuscular mycorrhizal fungus on the interaction of a binucleate *Rhizoctonia* species with Myc$^+$ and Myc$^-$ pea roots** *Mycorrhiza* **12**(2): 97–102 [*UMR BBCE-IPM, CMSE-INRA, BP 86510, 21065 Dijon Cédex, France]
Burke R M * and Cairney J W G. 2002	**Laccases and other polyphenol oxidases in ecto- and eri-coid mycorrhizal fungi** *Mycorrhiza* **12**(3): 105–116 [*Department of Biomolecular Sciences, UMIST, PO Box 88, Manchester, M60 1QD, UK]
Eriksen M*, Bjureke K E, and Dhilion S S. 2002	**Mycorrhizal plants of traditionally managed boreal grasslands in Norway** *Mycorrhiza* **12**(3): 117–123 [*Department of Biology and Nature Conservation, PO Box 5014, Agricultural University of Norway, 1432 Ås, Norway]
Malcová R*, Gryndler M, and Vosátka M. 2002	**Magnesium ions alleviate the negative effect of manganese on *Glomus claroideum* BEG23** *Mycorrhiza* **12**(3): 125–129 [* Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Pruhonice, Czech Republic]
Rangel-Castro J I*, Danell E, and Taylor A F S. 2002	**Use of different nitrogen sources by the edible ectomycorrhizal mushroom *Cantharellus cibarius*** *Mycorrhiza* **12**(3): 131–137 [*Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences (SLU), Box 7026, 750 07 Uppsala, Sweden]
Werner A*, Zadworny M, and Idzikowska K. 2002	**Interaction between *Laccaria laccata* and *Trichoderma virens* in co-culture and in the rhizosphere of *Pinus sylvestris* grown in vitro** *Mycorrhiza* **12**(3): 139–145 [*Department of Phytopathology, Institute of Dendrology, Polish Academy of Sciences, Parkowa 5, 62-035 Kórnik, Poland]

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]
Baar J*, Bastiaans T, van de Coevering M A, and Roelofs J G M. 2002	**Ectomycorrhizal root development in wet Alder carr forests in response to desiccation and eutrophication** *Mycorrhiza* **12**(3): 147–151 [*Applied Plant Research, Wageningen University Research, PO Box 6042, 5960 AA Horst, The Netherlands]
Orowska E* , Sz Z, Jurkiewicz A, Szarek-ukaszewska G, and Turnau K. 2002	**Influence of restoration on arbuscular mycorrhiza of *Biscutella laevigata* L. (Brassicaceae) and *Plantago lanceolata* L. (Plantaginaceae) from calamine spoil mounds** *Mycorrhiza* **12**(3): 153–160 [*Institute of Botany of the Jagiellonian University, ul. Lubicz 46, 31-512 Cracow, Poland]
Khasa P D*, Sigler L, Chakravarty P, Dancik B P, Erickson L, and McCurdy D. 2001	**Effect of fertilization on growth and ectomycorrhizal development of container-grown and bare-root nursery conifer seedlings** *New Forests* **22**(3): 179–197 [*Khasa PD, Universite Laval, Centre Recherche Biologie Forestiere, Pavillon Charles Eugene Marchand, Quebec City, PQ G1K 7P4, Canada]
Hurst S E*, Turnbull M H, and Norton D A. 2002	**The effect of plant light environment on mycorrhizal colonisation in field-grown seedlings of podocarp-angiosperm forest tree species** *New Zealand Journal of Botany* **40**(1): 65–72 [*Hurst S E, HortResearch, Private Bag 11030, Palmerston North, New Zealand]
Villenave C* and Duponnois R. 2002	**Interactions between ectomycorrhizal fungi, plant-parasitic and free-living nematodes and their effects on seedlings of the hardwood *Afzelia africana* Sm.** *Pedobiologia* **46**(2): 176–187 [*Villenave C, University Lyon 1, UMR CNRS 5557, F-69622 Villeurbanne, France]
Finnie C, Andersen C H, Borch J, Gjetting S, Christensen A B, deBoer A H, ThordalChristensen H, and Collinge D B*. 2002	**Do 14-3-3 proteins and plasma membrane H+-ATPases interact in the barley epidermis in response to the barley powdery mildew fungus?** *Plant Molecular Biology* **49**(2): 137–147 [*Collinge D B, Royal Veterinary and Agricultural University, Department of Plant Biology, Plant Pathology Sector, Thorvaldsensvej 40, DK-1871 Copenhagen C, Denmark]
Moynahan O S*, Zabinski C A, and Gannon J E. 2002	**Microbial community structure and carbon-utilization diversity in a mine tailings revegetation study** *Restoration Ecology* **10**(1): 77–87 [*Moynahan OS, University Montana, Division of Biological Science, Missoula, MT 59812 USA]
Stahl P D*, Perryman B L, Sharmasarkar S, and Munn L C. 2002	**Topsoil stockpiling versus exposure to traffic: A case study on in situ uranium wellfields** *Restoration Ecology* **10**(1): 129–137 [*Stahl PD, University Wyoming, Department of Renewable Resources, Laramie, WY 82071 USA]
Dahlberg A*.2002	**Effects of fire on ectomycorrhizal fungi in fennoscandian boreal forests** *Silva Fennica* **36**(1): 69–80 [*Dahlberg A, Swedish University of Agriculture Science, Department of Forest Mycology and Pathology, POB 7026, S-75007 Uppsala, Sweden]
Purdy B G*, Macdonald S E, and Dale M R T. 2002	**The regeneration niche of white spruce following fire in the mixedwood boreal forest.** *Silva Fennica* **36**(1): 289–306 [*Purdy BG, University of Alberta, Department of Renewable Resources, Edmonton, AB T6G 2E3, Canada]
Vij S P*, Lakhanpal T N, and Gupta A. 2002	**Orchidoid mycorrhiza and techniques to investigate** *Techniques in Mycorrhizal Studies* 385–434 [Vij SP*, Punjabi University, Department of Botany, Orchid Lab, Chandigarh 160014, India]

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]
Srivastava P S, Bharti N, Pande D, and Srivastava S. 2002	**Role of mycorrhiza in in vitro micropropagation of plants** *Techniques in Mycorrhizal Studies* 443–468 [*Srivastava P S, Hamdard University, Faculty of Science, Ctr Biotechnology, New Delhi 110062, India]
Hampp R* and Nehls U. 2001	**Physiology of tree root/fungus symbiosis** *Trends in European Forest Tree Physiology Research* **2**: 53–62 [*Hampp R, University Tubingen, Auf Morgenstelle 1, D-72076 Tubingen, Germany]
Motosugi H*, Yamamoto Y, Naruo T, Kitabayashi H, and Ishii T. 2002	**Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with an arbuscular mycorrhizal fungus *Gigaspora margarita*** *Vitis* **41**(1): 21–25 [*Motosugi H, Kyoto Prefectural University, University Farm, Seika, Kyoto 6190244, Japan]
ElSayed E S A*, ElDidamony G, and ElSayed E F. 2002	**Effects of mycorrhizae and chitin-hydrolysing microbes on *Vicia faba*** *World Journal of Microbiology & Biotechnology* **18**(6): 505–515 [*El-Sayed ESA, Zagazig University, Faculty of Science, Department of Botany, Zagazig, Egypt]
AbdelFattah G M and Shabana Y M*. 2002	**Efficacy of the arbuscular mycorrhizal fungus *Glomus clarum* in protection of cowpea plants against root rot pathogen** *Rhizoctonia solani Zeitschrift Fur Pflanzenkrankheiten und Pflanzenschutz - Journal of Plant Diseases and Protection* **109**(2): 207–215 [*Shabana YM, University of Mansoura, Faculty of Agriculture, Department of Plant Pathology, Mansoura 35516, Egypt]

Centre for Mycorrhizal Culture Collection

AMF biodiversity in wheat agrosystems of India

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Modern agriculture implies the simplification of the structure of the environment over vast areas, replacing nature's diversity with a small number of cultivated plants and domesticated animals (Altieri, 1999). The world's agricultural landscapes are planted mostly with 12 species of grain crops, 23 vegetable crop species, and about 35 fruit and nut crop species (Fowler and Mooney, 1990); i.e., no more than 70 plant species spread over approximately 1440 million ha of cultivated land in the world, a sharp contrast with the diversity of plant species found within 1 ha of a tropical rain forest, which typically contains over 100 species of trees (Perry, 1994). The type and abundance of biodiversity in agriculture differs across agroecosystems, which differ in age, diversity, structure and management. The degree of biodiversity in agroecosystems depends on four main characteristics of the agroecosystem (Southwood and Way, 1970).

- The diversity of vegetation within and around the agroecosystem.
- The permanence of the various crops within the agroecosystem.
- The intensity of management.
- The extent of the isolation of the agroecosystem from natural vegetation.

Since wheat is the staple food for much of the world, considerable scientific effort is aimed at yield improvement and more recently at yield

sustainability. In India, it is the second most important food crop next to rice and contributes about 25% to the total foodgrain production of the country. Knowing that the excessive use of chemical fertilizers is not a sustainable approach to increasing food grain production in the long run, alternative approaches are being sought. The potential for wheat yields to be improved under low-input agriculture by management of mycorrhizal symbiosis is therefore of considerable importance. It is evident from their effects upon soil health and host plant growth that AMF (arbuscular mycorrhizal fungi) are an important part of sustainable agricultural systems that have low inputs of chemical fertilizers and biocides (Bethlenfalvay and Schuepp, 1994, Jeffries and Barea, 1994; Hooker and Black, 1995). AMF improve water relations (Davies, Porter, and Linderman 1993), enhance nutrient uptake over non-mycorrhizal controls (George, Marschner, and Jakobsen 1995) and modify root morphology (Berta, Fusconi, Trotta, et al. 1990). The below-ground ecosystem as a whole is affected by AMF. These fungi are important in maintaining and enhancing the stability of soil aggregates (Tisdall and Oades, 1979; Miller and Jastrow 1990, 1992), affect nutrient cycling (Jeffries and Barea 1994) and carbon flow from the autotrophic plant to the heterotrophic soil microbial community because of their effect on root exudation (Graham 1985). This regulation of carbon flow can be an important regulator of the soil microbial community (Linderman 1991). For example, the presence of *Glomus mosseae* affected the relative abundance of rhizosphere bacteria species (Ames, Reid, and Ingham 1984). Modern, intensive agricultural practices such as chemical fertilization and pest control, continuous monoculture, and tillage, impact AMF and plant interactions. Describing the diversity of the community of the AMF at a site becomes, therefore, an important step in determining the effects of agricultural treatments upon AMF and the eventual development regimes for these fungi. Characterization of species-level diversity may provide the necessary basic information needed to assess the impact of soil and crop management practices on AMF communities and indirectly on some of the soil biological aspects of sustainable agricultural practices.

The CMR (Centre for Mycorrhizal Research) studied the diversity of AMF in 114 samples collected from 11 different wheat-growing regions of India. This study would then help in exploiting the potential of these fungi in wheat agricultural systems. The spore count, root colonization, species diversity and dominant species were found to vary with region as well as the soil nutrient conditions. A total of 33 species scattered over 5 genera were recovered. The genera *Glomus* was found to be ubiquitous. The distribution of other genera i.e. *Entrophospora, Gigaspora, Sclerocystis,* and *Scutellispora* was limited to only a few regions indicating the adaptability of genus *Glomus* to varied soil conditions. Other genera showed a narrow range of host-environment adaptation. Spores of *G. albidum* and *G. fasciculatum* were the most frequent ones (33% frequency) among the spore communities. Other species recorded include: *Entrophospora sp, Gigaspora gigantea, Gigaspora margarita. G. aggregatum, G. ambisporum, G. botryoides, G. caledonium, G. clarum, G. constrictum, G. dimorphicum, G. etunicatum, G. fulvum, G.intraradices, G. macrocarpum, G. microcarpum, G. monosporum, G. mosseae, G. mutisubtensum, G. pubescens, G. reticulatum, Sclerocystis coremoides, Scutellospora calospora, Scutellospora coralloides* and many other unidentified species. The wide host range of *Glomus* suggests that they are better competitors and are adapted to a wide range of soil conditions.

Although a total of 33 species was recovered from the fields, the number of species/field ranged only between 1 and 5. All the fields chosen for study were conventionally managed integrating the use of commercial seed-bed preparation, mechanized planting, chemical fertilizers and pesticides and requiring constant human intervention. These management-induced changes in abundance result in changes in habitat and substrate availability that may discourage the growth of selected microorganisms. Populations which survive the imposed stress, appear to have specific characteristics that enable them to persist within the perturbed community. The loss of species is generally in response to intense and widespread habitat modification (simplification) which causes local extinction of species.

Much has been written about the relationship between species diversity and ecosystem stability. A common view is that species diversity stabilizes ecosystem functional properties (McNaughton, 1977; Van Voris, O'Neill, Emanuel et al. 1980; Elliott and Lynch 1994). As agricultural systems are dynamic, an important issue in this diversity/stability relationship results in a better understanding of the role microbial communities have in the processes which support these systems. The diversity of soil microbial communities generally decreases in response to an environmental stress or disturbance which upsets the ecological balance of population interactions within the community (Atlas, Horowitz, Krichevsky, et al. 1991). It is likely that a system with high fungal diversity, with different fungi promoting plant biomass increment, plant survival and soil amelioration by aggregate formation, is more stable and more buffered against environmental and man-made disturbances than low-diversity systems. Such a fungal diversity is likely to be enhanced by diversity in habitat, diversity in host age and species within an ecosystem. On the other hand it is likely that under more stable conditions, a single effective endophyte will impart more benefits than would mixed inocula. As more information becomes available on the patterns of

biodiversity displayed by soil organism communities under various management regimes, it may become possible to correlate community composition with system stability. Knowledge of the diversity is required if the importance of biodiversity of AMF to the management and sustainability of agricultural systems is to be understood.

References

Altieri M A. 1999
The ecological role of biodiversity in agroecosystems
Agriculture, Ecosystems and Environment **74**: 19–31

Ames R N, Reid C P P, and Ingham E R. 1984.
Rhizosphere bacterial population responses to root colonization by a vesicular-mycorrhizal fungus
The New Phytologist **96**: 555–563

Atlas R M, Horowitz A, Krichevsky M, and Bej A K. 1991
Response of microbial populations to environmental disturbance
Microbial Ecology. **22**: 249–256

Berta G, Fusconi A, Trotta A, and Scannerini S. 1990
Morphogenetic modifications induced by the mycorrhizal fungus strain E3 in the root system of *Allium porrum* L.
The New Phytologist **114**: 207–215

Bethlenfalvay G J and Schüepp H. 1994
Arbuscular mycorrhizas and agrosystem stability
In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, pp. 117–131, edited by S Gianinazzi, H Schüepp
Basel, Switzerland: Birkhauser Verlag

Davies F T, Porter J R, and Linderman R G. 1993
Drought resistance of mycorrhizal pepper plants-independent of leaf phosphorus concentration, response in gas exchange, and water relations
Physiologia Plantarum 87: 45–53

Elliott L F and Lynch J M. 1994
Biodiversity and soil resilience
In *Soil Resilience and Sustainable Land Use*, pp. 205–230 edited by D J Greenland, I S Szabolcs
New York: John Wiley & Sons

Fowley C and Mooney P. 1990
Shattering: Food, politics and the loss of genetic diversity
In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, pp. 101–115, edited by S Gianinazzi, H Schüepp
Basel, Switzerland: Birkhauser Verlag

George E, Marschner H, and Jakobsen I. 1995.
Role of arbuscular mycorrhizal fungi in uptake of phosphorus and nitrogen from soil.
Critical Reviews in Biotechnology **15**: 257–270

Graham J H. 1985
Membrane regulation of mycorrhizal symbiosis, pp 155–156
Paper presented at the *Proceedings of the 6th North American Conference on Mycorrhizae*, Bend, OR, USA, 25–29 June 1984

Hooker J E, Black K E. 1995
Arbuscular mycorrhizal fungi as components of sustainable soil-plant systems
Critical Reviews in Biotechnology **15**: 201–212

Jeffries P and Barea J M. 1994.
Biochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systems
In *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems*, pp. 101–115, edited by S Gianinazzi, H Schüepp
Basel, Switzerland: Birkhauser Verlag

Linderman R G. 1991
Mycorrhizal interactions in the rhizosphere
In *The Rhizosphere and Plant Growth*, pp. 343–348, edited by D L Keister, P B Cregon
The Netherlands: Kluwer Academic Publishers

McNaughton S J. 1977.
Diversity and stability of ecological communities: A comment on the role of empiricism in ecology
Am. Nat. **111**: 515–525

Miller R M and Jastrow J D. 1990
Hierarchy of root and mycorrhizal fungal interactions with soil aggregation
Soil Biology and Biochemistry **22**: 579–584

Miller R M and Jastrow J D. 1992
The role of mycorrhizal fungi in soil conservation
In *Mycorrhizae in Sustainable Agriculture*, pp. 24-44, edited by G J Bethlenfalvay, R J Linderman
Madison, WI: Agron. Soc. Am. Special Publication, No. 54.

Perry D A. 1994
Forest Ecosystems
Baltimore, MD: Johns Hopkins University Press, 649 pp.

Southwood R E and Way M J. 1970
Ecological background to pest management
In *Concepts of pest management*, pp. 6–29, edited by R C Rabb, F E Guthrie
Raleigh, NC: North Carolina State University

Tisdall J M and Oades J M. 1979
Stabilization of soil aggregates by the root systems of ryegrass.
Aust. J. Soil Research **17**: 429–441

Van Voris P, O'Neill R V, Emanuel W R, and Shugart H H Jr. 1980
Functional complexity and ecosystem stability
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