Vol. 14 No. 2
July 2002

About TERI

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

The Bioresources and Biotechnology Division

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

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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

Contents

Role of Mycorrhiza in plants raised from cuttings or in micropropagated plants. Part IV. Crops bearing edible fruits and root tubers

*Sujan Singh**
TERI, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi – 110 003, India

Mycorrhizal association has been found to enhance rooting, and promote the growth of plants raised from cuttings or by tissue culture belonging to crops bearing edible fruits. These are discussed below.

Actinidia deliciosa (kiwifruit)

Plants raised from cuttings

In studies conducted at the Pukekohe Horticultural Research Station, MAF, Pukekohe, New Zealand, kiwi fruit (*Actinidia deliciosa* var. *deliciosa*) cuttings were grown in peat-pumice mix (amended with three rates of phosphorus fertilizer and five rates of mycorrhizal inoculum) for 130 days in a heated glasshouse. Phosphorus fertilizer increased shoot length and weight by up to 170% and 145%, respectively. Mycorrhizal inoculation increased shoot length and weight by 294% and 265%, respectively, with no significant interaction between inoculation and fertilizer (Powell 1986).

On micropropagated plants

In studies conducted at the Centro Miglioramento Genetico e Biologia Vite, CNR, Grugliasco, micropropagated shoots of kiwifruit (*Actinidia deliciosa*) were rooted in media containing a vital staining dye in order to distinguish in vitro from in vivo-formed roots. At transfer to soil, plants were inoculated with the VAM fungus *Glomus mosseae*. The presence of the dye did not reduce plant growth and also did not prevent root colonization by the VAM fungus. Four to five weeks after transplanting, both in vitro and in vivo-formed roots were colonized by the VAM fungus (Gribaudo, Zanetti, Morte et al. 1996).

Alocasia sp.

On micropropagated plants

Studies conducted at the Kerala Agricultural University, College of Agriculture, Vellayani, India, on the effect of AM fungi (*Glomus fasciculatum*, *G. etunicatum* and a native *Glomus* sp.) on tissue culture plantlets of *Alocasia* sp. showed that AM colonization significantly increased the establishment rate of *Alocasia* from 73.6 to 100%. There was a significant increase in growth vigour and biomass production during acclimatization and after transplanting to pots when inoculated with *Glomus* sp. and *G. fasciculatum*. *G. fasciculatum* colonization increased the P and Zn content remarkably (Sivaprasad, Suneetha, Joseph, et al. 1999).

Ananas comosus (pineapple)

On micropropagated plants

Studies were conducted at the Station de Genetique et Amelioration des Plantes, INRA, Dijon, France, to evaluate the effect of VAM inoculation on micropropagated plantlets of two pineapple clones, inoculated with *Glomus* sp. or with *G. intraradices*, and grown in acid or alkaline soils with application of a nutrient solution with or without phosphorus. VAM inoculation increased the growth of plants as measured by leaf number, fresh and dry weight of aerial parts or roots and leaf area. The effect was greater in plants not given phosphorus and those grown in acid soils. Also, VAM inoculation resulted in larger and more efficient root systems. Application of phosphorus increased growth of uninoculated plants, reduced the effect of inoculation in acid soils and prevented the

* Compiled from TERI database – RIZA

effect in alkaline soils (Guillemin, Gianinazzi, and Gianinazzi-Pearson 1991).

Studies conducted at the Departamento de Proteccion Vegetal, Centro de Investigacion y Tecnologia Agraria, Apartado, La Laguna, Tenerife, Canary Islands, showed that in greenhouse experiments, inoculation of micropropagated pineapple plantlets (cv. Smooth Cayenne) with *Acaulospora* sp., *Glomus mosseae*, and *G. fasciculatum* increased survival of the transplants by 100%, 80% and 80% respectively, compared with 40% in uninoculated controls. VAM inoculation also increased biomass production and N, P, and K levels (JaizmeVege and Azcon 1991).

Studies conducted at the INRA, CNRS, Phytoparasitol Laboratory, Genet and Ameliorat Plantes, Dijon, France, on micropropagated plants of pineapple (*Ananas comosus*) cv. Queen Tahiti and Smooth Cayenne, inoculated at transplanting from axenic conditions with a VAM fungus showed that the growth and mineral nutrition of VAM plants were not affected by different inoculum levels of the root-rot pathogen *Phytophthora cinnamomi*, while these parameters were reduced for non-mycorrhizal plants. Root/shoot ratios of VAM plants was lower than that of non-mycorrhizal plants and the pathogen did not modify this effect except at the highest inoculum levels of *P. cinnamomi*. VAM colonization was not altered by the pathogen (Guillemin, Gianinazzi, Gianinazzi-Pearson et al. 1994).

Studies conducted at the EMBRAPA-CNPAB, Itaguaim, Brazil, on micropropagated pineapple inoculated with *Glomus clarum*, *Gigaspora margarita* and *Acaulospora* sp. showed that during acclimatization in the greenhouse, no differences in growth (height and vigour) were observed between VAM-inoculated and non-VAM (control) treatments. Six months after transplanting in the field, plants inoculated with VAM in the greenhouse were taller than the uninoculated control plants (Matos and da Silva 1996).

Fragaria ananassa (strawberry)

On micropropagated plants

Studies conducted at the Phytopathology and Soil Microbiology Division, Swiss Federal Research Station, Wadenswil, Switzerland, showed that non-mycorrhizal micropropagated strawberry plants produced more runners than mycorrhizal plants under controlled growth conditions (Phytotron). Transfer of potted plants to the field resulted in drastic alterations in overall growth and development within four weeks. Mycorrhizal plants became healthy, produced many stout runners and in var. *elsanta*, even produced more runner than the control. However, in var. *avanta* the number of runners and their biomass was almost the same as in the control (Varma and Schuepp 1994).

In studies conducted at the Centre de Recherche en Horticulture, Faculte des Sciences de l'Agriculture et de l'Alimentation, Universite Laval, Quebec, Canada, an in vitro system for culturing the VAM fungus, *Glomus intraradices* with Ri t-DNA-transformed carrot root or non-transformed tomato roots was used as a potential active source of inoculum for the colonization of micropropagated strawberry plantlets. The plantlets grown in cellulose plugs were placed in contact with the primary mycorrhizae in growth chambers enriched with 5000 ppm CO_2 and fed with a minimal medium. After 20 days, all plantlets were colonized by the VAM fungus. As a source of VAM inoculum, 30-day-old transformed carrot roots had a substantially higher infection potential than 5, 10 or 20 day-old VAM. Colonized plantlets had more extensive root systems and better root growth than control plants. Also, VAM symbiosis reduced the plantlet osmotic potential (Elmeskaoui, Damont, Poulin et al. 1995).

In studies conducted at the Agriculture Research Centre, Laukaa Research and Elite Plant Unit, Laukaa, Finland, micropropagated strawberry cv. Jonsok, susceptible to the disease caused by *Phytophthora cactorum*, was inoculated or not with *Glomus mosseae* (Finnish strain), *G. hoi*, and *G. fistulosum*. AM fungal inoculation at the beginning of the weaning stage, five weeks before the establishment of the pot or field experiment, did not decrease crown root-rot severity in either of the experiments. In the pot experiment, however, AM fungi lowered the plant health index when *P. cactorum* was added to the substrate in the form of infected plant residues (Vestberg, Palmujoki, Parikka et al. 1994).

Studies conducted at the New Mexico State University, Las Cruces, USA, on micropropagated strawberry (*Fragaria ananassa*) plantlets showed that under in vitro conditions of high humidity, relative water content of the complete plant was 11% higher in AM-(*Glomus intraradices*) colonized plantlets compared to non-AM plantlets. However, root and leaf osmotic potential, leaf stomatal conductance, and relative water content of leaf discs were not affected by the fungal inoculation. The significant increase of relative water content of whole plants was related neither to improved mineral nutrition nor growth stimulation of mycorrhizal plantlets (HernandezSebastia, Piche and Desjardins 1999).

In studies conducted at the Seccion de Microbiologia, Centro de Edafologia, Collegio de Postgraduados, Montecillos, Mexico, the response of micropropagated strawberry cultivars Douglas, Tioga, Aiko, and Pajaro to colonization by three VAM fungi was determined under nursery conditions. VAM fungi used in the experiment were *Glomus* sp. CPH-23, *G. macrocarpum*, and *G. versiforme*. Yield in VAM plants tended to exceed that of non-VAM plants during the latter part of

the harvesting season but VAM effects differed widely with host-endophyte combination. The cultivar-endophyte combinations which produced the best yields were Douglas and Glomus sp. CPH-23 (78.7 g/plant), Tioga and *G. macrocarpum* (80.9 g/plant) and Aiko and *G. versiforme* (96.8 g/plant). In Pajaro, non-VAM plants and plants colonized by *G. macrocarpum* produced the best yields (84.9 and 63.0 g/plant, respectively). The number of fruits/plant differed significantly in Tioga depending on the endophyte used. Root colonization by VAM fungi varied from 25-75%. Plant growth was not related to the extent of fungal colonization (Chavez and Ferrera-Cerrato 1990).

Manihot esculenta (cassava)

Cuttings

In studies conducted at the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, USA, cassava (*Manihot esculenta*) plants were raised in the greenhouse either from small cuttings (2.0 mg P/cutting) or large cuttings (20.2 mg P/cutting) in a subsurface oxisol and were inoculated or not inoculated with *Glomus aggregatum* at target soil solution P concentrations of 0.003-0.2 mg per litre. VAM colonization exceeded the 60% level irrespective of cutting size or soil P level. Plants from large cuttings grew faster than those from small cuttings. The latter were very highly dependent on mycorrhiza while large cutting plants were only marginally dependent. This appears to be related to the high P reserve in large cuttings and hence low requirement of soil P by the plant until the P reserve in the cuttings is significantly depleted (Habte and Byappanahalli 1994).

On micropropagated plants

In studies conducted at the CSIC, Estacion Experimental Zaidin, Department Microbiology, Suelo and Systemas Simbiot, Granada, Spain, about 90% of cassava (*Manihot esculenta*) plantlets were successfully rooted in vitro and 75% survived after the acclimatization stage. Inoculation with *Glomus deserticola* early in the post vitro, weaning stage enhanced percent survival and improved tolerance to the transplanting stress. Shoot, root and tuber development of the micropropagated plants was increased by VAM inoculation but growth responses were dependent on both the cultivar (clone) and the AM fungi involved. *G. deserticola* was very effective in improving the growth of both tested clones while the effectiveness of *G. clarum* and *C. fasciculatum* was dependent on the clone (AzconAguilar, Cantos, Troncoso et al. 1997).

Studies conducted at the Biologo, Ph D Institute Agronomico do Parana, Londrina, Bolsista, Brazil, on micropropagated cassava plants showed that coinoculation of VAM fungi and bacterium E increased shoot and root dry weights up to 50% and 105%, respectively, in relation to the bacterium alone. This coinoculation also increased shoot and root nitrogen upto 88% and 173% and phosphorus upto 83% and 158%, respectively in relation to the bacterium alone. Coinoculation of bacterium E with *Glomus clarum* also increased mycorrhizal colonization by 40% and sporulation up to 168% in comparison to the fungi alone (Balota, Lopes, Hungria et al. 1997).

Musa acuminata (banana)

On micropropagated plants

In studies conducted at the Instituto Agronomico per l'Oltremare, Florence, Italy, micropropagated bananas (*Musa acuminata*) plants were grown on a sterilized medium (1:1 mixture of soil and sand containing 6 ppm of $NaHCO_3$-extractable phosphorus) and inoculated with *Glomus mosseae* or *G. monosporum*. VAM infection enhanced growth, phosphorus, and nitrogen uptake and biomass production. *G. mosseae* appeared to be more effective as a symbiont than *G. monosporum*, probably due to a higher level of colonization of root cortex and arbuscular development. Comparison between growth response to VAM inocula and to application of 33 ppm KH_2PO_4 fortnightly in relation to shoot tissue phosphorus concentration suggested that VAM effectiveness was not exclusively due to enhanced phosphorus uptake, and this was confirmed when shoot phosphorus concentrations were plotted against relative leaf expansion rate (Rizzardi 1990).

Studies conducted at the INRA, Station d'Agronomie, Dijon, Cedex, France, showed that inoculation of micropropagated banana plants (cv. AAA Giant Cavendish) with *Glomus mosseae* and *G. geosporum* in two complementary experiments resulted in greater fresh and dry weights of shoots and higher phosphorus and potassium contents, particularly for *G. mosseae*. Differences in infection development (high proportion of plants infected by *G. mosseae*) were related to different growing conditions, particularly soil pH (Declerck, Devos, Delvaux, et al. 1994).

In studies conducted at the Pusat Penelitian, Kopi dan Kakao, PB Sudirman, Jember, Indonesia, banana (*Musa* sp.) plantlets were cultured in vitro on a medium containing 85 or 170 mg KH_2PO_4 per litre, 75 or 150 mg Ca-phytate (organic phosphorus source) per litre or 85 mg KH_2PO_4+75 mg Ca-phytate per litre and inoculated with *Gigaspora margarita.* A moderate rate of infection occurred on the medium containing 75 mg Ca-phytate per litre and a low rate occurred on that containing 170 mg KH_2PO_4 per litre. No infection occurred on the other media, including controls containing no added phosphorus (Winarsih, Baon, and Priyono 1995).

Studies conducted at the Kerala Agriculture University, College of Agriculture, Vellayani, India, on the effect of AM fungi (*Glomus fasciculatum*, *G. etunicatum* and a native *Glomus* sp.) on tissue

culture plantlets of banana showed that AM colonization significantly increased the establishment rate of banana from 68.5 to 92%. There was a significant increase in growth vigour and biomass production during acclimatization and after transplanting to pots, when inoculated with *Glomus* sp. and *G. fasciculatum*. *G. fasciculatum* colonization remarkably increased the P and Zn content (Sivaprasad, Suneetha, Joseph et al. 1999).

Studies conducted at the Department of Horticulture, National Taiwan University, Taiwan, showed that roots of micropropagated banana plantlets could be colonized by all the three *Glomus* spp. tested (*G. mosseae*, *G. fasciculatum*, *G. etunicatum*). Four months after inoculation, 80% of roots were colonized. The growth of VAM colonized banana plantlets in autoclaved growth media showed significantly higher total dry weights, pseudostem diameters, and plant heights than those in non-autoclaved culture media. The growth of mycorrhizal plants was always better than non-inoculated controls. All root tissues except the root cap, stele, and endodermis could be infected with VAM fungi (Lin and Chang 1987).

Persea americana (avocado)

On micropropagated plants

Studies conducted at the Departmento de Microbiologia, Estacion Experimental del Zaidin, Granada, Spain, showed that inoculation of micropropagated plantlets of avocado with *Glomus fasciculatum* improved the formation of a well-developed root system that was converted into a mycorrhizal system. Introduction of the mycorrhizal fungus at the time plantlets were transferred from axenic conditions to ex vitro conditions, improved shoot and root growth, increased the shoot-root ratio, the concentration and/or content of nitrogen, phosphorus, and potassium in plant tissues, and helped plants to tolerate environmental stress at transplanting. Inclusion of soil as a component of the potting medium appeared to favour mycorrhiza formation and effectiveness (Vidal, AzconAguilar, Barea, et al. 1992).

Rubus sp. (raspberry)

Studies conducted at INRA, Laboratoire de Phytoparasitologie, Station d'Amelioration des Plantes, Dijon, Cedex, France, showed that irrespective of the culture condition (growth room, glasshouse), growth of raspberry plants was better when they were inoculated with a mycorrhizal fungus at the moment of transplant from the culture tubes into the soil. Mycorrhizal infection was 80%–90%. Mycorrhizal raspberries were more vigorous and more uniform in size than the non-mycorrhizal controls. Shoot dry weight production of the variety 'Bios Blanc' transplanted in an acid soil was increased by 71% when inoculated with *Glomus tenuis*. Shoot dry weight increases for the variety 'Nygymarosi', cultivated in a neutral soil were 53% and 130% after inoculation with *G. mosseae* and *G. fasciculatum*, respectively. Increased NK fertilization improved this mycorrhizal effect and growth compared to non-mycorrhizal controls (Morandi, Gianinazzi and Gianinazzi-Pearson 1979).

Vaccinium angustifolium (blueberry)

On micropropagated plants

In studies conducted at the Department of Plant and Soil Science, University of Maine, Oreno, USA, tissue culture plantlets of *Vaccinium angustifolium* (Clone NB-3), rooted in a peat/perlite/vermiculite medium, were grown in Can-Am containers. The plantlets were inoculated with macerated mycelium (from plates of modified Melin-Norkrans medium) of two native and one England isolate of *Hymenoscyphus ericae* and were fertilized or not with the slow release fertilizer, Osmocote. Height and branching were noted after 10 weeks of greenhouse growth and dry weight measured after 22 weeks. Fertilizer increased height, branching, dry weight and total nitrogen. Mycorrhizal infection levels were significantly less in the fertilized plantlets than in the unfertilized ones. In similar experiments with clones ME-3, 723, and Chignecto, fertilizer did not suppress colonization, but results for growth and nitrogen content were essentially the same as in clone NB-3 (Smagula and Litten, 1989).

Studies were conducted at the Department of Horticulture, Pennsylvania State University, University Park, USA, to determine the effect of aluminium (0 and 600 µM) and media (sand and sand+soil in 1:1 ratio) on ericoid mycorrhizal and non-mycorrhizal plantlets of blueberry (*Vaccinium corymbosum*). There was no difference in nutrient uptake and total plant dry weight between mycorrhizal and non-mycorrhizal plantlets though more root growth, as determined by dry weight, was observed in the mycorrhizal plantlets. Plantlets growing in sand had a greater dry weight than those growing on soil medium. The root and shoot growth and nutrient uptake were reduced by 600 µM Al but the direct effect of Al on growth of plantlets was not clear due to Al and P interactions. Mycorrhizal cortical cell infection levels of 15–20% were maintained in the roots in soil medium but decreased to about 5% over the six weeks of the experiment in sand. Although mycorrhizal plantlets accumulated more Al in their roots, it was readily transported to the leaf tissues of mycorrhizal and non-mycorrhizal plantlets (Yang, Goulart, and Demchak 1996).

Further studies conducted at the above university on *Vaccinium corymbosum* plantlets, grown under plastic tents and inoculated with ericoid mycorrhizal fungi belonging to the genera *Hymenoscyphus*, *Oidiodendron* and *Scytalidum*, showed that many of the negative effects of Al

characterized by decreased shoot, root and total plant dry mass were reversed by foliar P and N application, indicating that the P and N uptake were limited by high Al concentration. However, Al-mediated growth reduction in P-stressed plants indicated that the restriction of P uptake by high Al may not have been the only mechanism for Al toxicity in this experiment. Root Al and P concentrations were negatively correlated in non-mycorrhizal plantlets but this was not so in mycorrhizal plants, suggesting that mycorrhizal infection may alter P uptake processes. More Al accumulated in mycorrhizal than in non-mycorrhizal plant roots and leaves (Yang and Goulart 1997).

Vitis sp. (grapevine)

On plants raised from cuttings

Studies were conducted at the Dipartimento di Ortoflorofrutticoltura, Universita di Firenze, Firenze, Italy, on interactions between VAM (vesicular-arbuscular mycorrhiza) inoculation with *Glomus constrictus*, *G. deserticola,* and *G. mosseae* and soil lime content (0, 5, 10, 15, 20, 25 or 30 per cent active lime) and their effects on the growth of rooted grapevine (Kober 5BB) cuttings. Overall, mycorrhizal plants had greater root and shoot growth than the control (without VAM inoculation) irrespective of soil lime content. *G. mosseae* and *G. constrictus* caused greater vegetative development than *G. deserticola*. However, plant growth was reduced by increasing soil lime content. Mycorrhization, while increasing vine growth, did not reduce grapevine susceptibility to calcium carbonate. Leaf chlorosis was found to be significantly higher in leaves of the inoculated cuttings; its incidence was greater at higher liming rates. Leaf chlorophyll content (µg/mg) was reduced by VAM, while chlorophyll content per leaf unit area was also inversely correlated to lime concentration in the substrate. Leaf mineral content was not influenced by soil lime, except for Mg which decreased with increased lime concentration. Inoculation with VAM fungi influenced the mineral content of phosphorus, manganese and boron, and to a lesser extent, zinc and copper (Biricolti, Ferrini, Rinaldelli, et al. 1997).

In studies conducted at the Institute di Frutti-Viticulture, Universite Cottolica S, Cuore, Piacenza, Italy, one-year old rooted cuttings of grapevine cv. Pinot blanc clone 55, grafted on SO_4 or 41B hybrid root stock, were infected by either *Glomus mosseae,* or with a suspension of the bacteria *Erwinia* sp. or *Pseudomonas fluorescens* or *Enterobacter cloacae* and then grown in pots containing a calcareous soil. Root infection with *Erwinia* sp. or *P. fluorescens* increased the chlorophyll concentration over untreated controls in both graft combinations. Plants grafted on SO_4 (which is less lime tolerant than 41B) benefited from *G. mosseae* treatment. Root inoculation with *E. cloacae* depressed shoot growth and chlorophyll concentration in both graft combinations (Bavaresco and Fogher 1996).

In studies conducted at the Indian Institute of Horticultural Research, Hessaraghatta Lake, Bangalore, India, rooted cuttings of grape cultivars were inoculated with *Aureobasidium pullulans* by dipping the roots in washed mycelium and conidia of *A. pullulans* grown in modified Melin Norkrans broth and mixed with a carrier (farmyard manure vermiculite and moss). The inoculum was also applied as a band around the root zone before transplanting. Fifteen days after transplanting, bud sprouting was observed in inoculated plants. Five months after transplanting, the inoculated fungus was seen as a weft of mycelium on 10% of the roots examined and showed no typical ectomycorrhizal sheath and Hartig net. However, increased branching and elongation of roots was observed. The inoculated plants showed significant increases in shoot and root dry weight and phosphorus levels in leaves over uninoculated plants (Onkarayya and Mohandas 1987).

On micropropagated plants

In studies conducted at the Institute of Forestry, Chines Academy of Forestry, Wan Shou Shan, Beijing, China, 30-day old tissue cultured grapes were inoculated with *Glomus epigaeus* during the transplant from tubes to containers. Arbuscules were found in cortical cells of roots at three weeks after inoculation. At six weeks, these arbuscules began to collapse and a lot of vesicles were produced inter and intra cellularly. Shoot height and root collar diameter were greatly increased by VAM inoculation over non-inoculated controls. Inoculated grape plantlets were transplanted from containers to nurseries after 45 days of inoculation where they maintained their growth advantage over the non-inoculated control inspite of their more serious infection by *Plasmopara viticola* diseases than the control. Higher disease infection of inoculated grape vine was due to higher total saccharide content of leaves than control plants (Kuo and Li 1987).

In studies conducted at the Dipartimento di Biologia Vegetale, Universita di Torino, Turin, Italy, endomycorrhizal formation in micropropagated plantlets of *Vitis vinifera* (cv. $SO_4$102) caused increases in lateral root number and consequently total root length, but did not alter the number of root axes. The rate of production of any order lateral roots was higher in mycorrhizal than in non-mycorrhizal controls. The number of first- and second-order laterals increased linearly with time in mycorrhizal plants and fitted a logistic function in the controls. Topological analysis indicated similar patterns of root branching in the early stages of growth, but the root system of non-mycorrhizal plants adopted a herring bone pattern after eight weeks (Schellenbaum, Berta, Ravolanirina, et al. 1991).

Studies conducted at the INRA, Laboratoire de Phytoparasitologie, Station d'Amelioration des

Plantes, Dijon, Cedex, France, on micropropagated grapevine rootstocks showed that inoculation with *Glomus mosseae*, *G. caledonium*, *G. fasciculatum* or *Gigaspora margarita* in vitro resulted in greater growth stimulation compared with uninoculated controls, and this continued after transplanting. However, inoculation at the time of transplanting was much more effective in stimulating growth. An increase in phosphorus supply in the potting mixtures reduced the response to mycorrhizal infection (Ravolanirina, Gianinazzi, Trouvelot, et al. 1990).

In studies conducted at the Centro Miglioramento Genetico e Biologia Vite, CNR, Via Leonardo da Vinci, Grugliasco, micropropagated shoots of grapevine (*Vitis berlandieri* x *V. riparia* Kober 5 BB), were rooted in media containing vital staining in order to distinguish in vitro- formed roots from in vivo-formed roots. Presence of the dye did not reduce plant growth. At transfer into the soil, plants were inoculated with the VAM fungus, *Glomus mosseae*. The dye did not prevent root colonization by the VAM fungus. Four to 5 weeks after transplanting, both in vitro- and in vivo-formed roots were colonized by the VAM fungus (Gribaudo, Zanetti, Morte et al. 1996).

Oil palms

On micropropagated plants

In studies conducted at the INRA, Laboratoire de Phytoparasitologie, Station d'Amelioration des Plantes, Dijon, Cedex, France, indigenous VA mycorrhizal fungi, isolated from two tropical acid soils of oil palm plantations were used to pre-inoculate micropropagated oil palm explants before planting. Mycorrhizal establishment significantly improved oil palm growth and mineral nutrition. Similar effects could be achieved in non-mycorrhizal oil palm explants by adding soluble phosphate to cultures but different clones obtained by micropropagation showed variability in the phosphorus uptake capacity of roots. In contrast, inoculation with efficient indigenous VA mycorrhizal fungi suppressed this variability among clones and improved the growth of all the oil palm clones to a similar extent (Blal and Gianinazzi-Pearson 1988).

In studies conducted at the Indonesian Biotechnology Research Institute for Estate Crops, Boger, Indonesia, micropropagated oil palm plantlets of the cultivars MK58 and MK65 were inoculated with *Acaulospora delicata*, *Glomus fasciculatum* or *Entrophospora colombina*, transplanted into an ultisol soil two months later and were acclimatized in a greenhouse. After nine months, plant growth of MK58 (across all treatments) was greater than MK65. Mycorrhizal inoculation increased plant growth parameters and in some cases, increased the uptake of P, K, Ca, and Mg when compared with non-inoculated control plants. The greatest increases were obtained with *E. colombiana* which increased leaf, stem, and root dry weight by 44%, 45% and 47%, respectively and plant height by 13% when compared with controls. (Widiastuti and Tahardi 1993).

Coffee

On micropropagated plants

Studies conducted at the Pusat Penelitian Kopi dan Kakao, Jember, Indonesia, showed that in experiments on modified MS medium, the addition of P in various forms and concentrations and inoculation with the mycorrhiza fungus, *Gigaspora margarita*, had no effect on the growth in coffee clone BP 42 plantlets in vitro. The degree of mycorrhizal infection was greater in plantlets grown with a single P source than in those grown with a combination of P sources (Winarsih 1996).

References

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

AzconAguilar C, Cantos M, Troncoso A, and Barea J M. 1997
Beneficial effect of arbuscular mycorrhizas on acclimatization of micropropagated cassava plantlets
Scientia Horticulturae **72**(1): 63–71

Balota E L, Lopes E, Hungria M, and Dobereiner J. 1997
Inoculation of diazotrophic bacteria and arbuscular mycorrhizal fungi on the cassava crop
Pesquisa Agropecuaria Brasileira **32**(6): 627–639

Bavaresco L and Fogher C. 1996
Lime-chlorosis occurrence and leaf mineral composition of grapevine treated by root microorganisms
Journal of Plant Nutrition **19**(1): 87–98

Biricolti S, Ferrini F, Rinaldelli E, Tamantini I, and Vignozzi N. 1997
VAM fungi and soil lime content influence rootstock growth and nutrient content
American Journal of Ecology and Viticulture **48**(1): 93–99

Blal B and Gianinazzi-Pearson V. 1988
Interest of endomycorrhizae for the production of micropropagated oil palm clones, p.12
In *Proceedings of the Second European Symposium on Mycorrhizae*
Prague: Czechoslovak Society for Microbiology, 115 pp.
[Prague, Czechoslovakia, 14–20 August 1988]

Chavez M G and Ferrera-Cerrato R. 1990
Effect of vesicular-arbuscular mycorrhizae on tissue culture-derived plantlets of strawberry
HortScience **25**(8): 903–905

Declerck S, Devos B, Delvaux B, and Plenchette C. 1994
Growth response of micropropagated banana plants of VAM inoculation
Fruits (*Paris*) 49(2): 103–109

Elmeskaoui A, Damont J P, Poulin M J, Piche Y, and Desjardins Y. 1995
A tripartite culture system for endomycorrhizal inoculation of micropropagated strawberry plantlets in vitro
Mycorrhiza **5**(5): 313–319

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

Guillemin J P, Gianinazzi S, and Gianinazzi-Pearson V. 1991
Endomycorrhization of vitroplants of *Ananas comosus*: demonstration of a mycorrhizal effect
Fruits (Paris) **46**: 355–358

Guillemin J P, Gianinazzi S, Gianinazzi-Pearson V, and Marchal J. 1994
Contribution of arbuscular mycorrhizas to biological protection of micropropagated pineapple (*Ananas comosus* (L.) Merr.) against *Phytophthora cinnamomi* Rands.
Agricultural Science in Finland **3**(3): 241–251

Habte M and Byappanahalli M N. 1994
Dependency of cassava (*Manihot esculanta* Crantz) on vesicular-arbuscular mycorrhizal fungi
Mycorrhiza **4**(6): 241–245

HernandezSebastia C, Piche Y, and Desjardins Y. 1999
Water relations of whole strawberry plantlets in vitro inoculated with *Glomus intraradices* in a tripartite culture system
Plant Science **143** (1): 81–91

JaizmeVege M C and Azcon R. 1991
Effect of vesicular-arbuscular mycorrhizal fungi on pineapple (*Ananas comosus* (L.) Merr.) in the Canary Islands
Fruits (Paris) **46**(1): 47–50

Kuo S C and Li Z S. 1987
Effects of VA mycorrhizae on the growth of tissue cultured grapes
In *Proceedings of the Seventh North American Conference on Mycorrhiza: Program and abstracts,* p. 51, edited by Sylvia D M, Hung L L and Graham J H
Florida: University of Florida, 77 pp.
[Gainesville, Florida, 3–8 May 1987]

Lin C H and Chang D C N. 1987
Effect of three Glomus endomycorrhizal fungi on the growth of micropropagated banana plantlets
Transactions of the Mycological Society (China) **2**(1): 37–45

Matos R M B and Silva da E M R. 1996
Effect of inoculation by arbuscular mycorrhizal fungi on the growth of micropropagated pineapple plants
Fruits(Paris) **51**(2): 115–119

Morandi D, Gianinazzi S and Gianinazzi-Pearson V. 1979
Interet de l'endomycorhization dans la reprise et la croissane du frasmboisier issu de multiplication vegetative in vitro. Interest of using endomycorrhizae for the establishment and growth of axenically propagated raspberry plants after transplanting
Annales de l'Amelioration des Plantes **29**(6): 623–630

Onkarayya H and Mohandas S. 1987
Preliminary studies on the influence of an ectomycorrhizal fungus *Aureobasidium pullulans* (de Bary) arnaud on grapes, pp. 124–128
In *Mycorrhiza Round Table*, edited by Verma A K, Oka A K, 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]

Powell C V. 1986
Effect of mycorrhizal inoculation and phosphorus fertilizer on the growth of hardwood cuttings of kiwifruit (*Actinidia deliciosa* cv. Hayward) in containers
New Zealand Journal of Agriculture Research **29**: 263–68

Ravolanirina F, Gianinazzi S, Trouvelot A and Carre M. 1990
Production of endomycorrhizal explants of micropropagated grapevine rootstocks
Agriculture, Ecosystems and Environment **29**(1–4): 323–327

Rizzardi V. 1990
Effect of inoculation with vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated *Musa acuminata* clone 'Grand Nain'
Rivista di Agricoltura Subtropicale e Tropicale **84**(3): 473–484

Sivaprasad P, Suneetha S, Joseph P J, Sulochana K K, and Rajmohan K. 1999
Influence of arbuscular mycorrhizal fungi (AMF) on establishment and growth of tissue culture plantlets of banana and alocasia
In *Proceedings of the National Conference on Mycorrhiza*: Association and distribution of mycorrhizal fungi under varying edapho-climatic conditions.
Bhopal: India
[Bhopal, India, 5–7 March 1999]

Schellenbaum L, Berta G, Ravolanirina F, Tisserant B, Gianinazzi S, and Fitter A H. 1991
Influence of endomycorrhizal infection on root morphology in a micropropagated woody plant species (*Vitis vinifera* L.)
Annals of Botany **68**(2):135–141

Smagula J M and Litten W. 1989
Effect of ericoid mycorrhizae isolates on growth and development of lowbush blueberry tissue culture plantlets
Acta Horticulturae **241**: 110–113

Varma A and Schuepp H. 1994
Infectivity and effectiveness of *Glomus intraradices* on micropropagated plants
Mycorrhiza **5**(1): 29–37

Vestberg M, Palmujoki H, Parikka P, and Uosukainen M. 1994
Effect of arbuscular mycorrhizas on crown rot (*Phytophthora cactorum*) in micropropagated strawberry plants
Agricultural Science in Finland **3**(3): 289–295

Vidal M T, AzconAguilar C, Barea J M, and Pliego-Alfaro F. 1992
Mycorrhizal inoculation enhances growth and development of micropropagated plants of avocado
HortScience **27**(7): 785–787

Widiastuti H and Tahardi J S. 1993
Effect of vesicular-arbuscular mycorrhizal inoculation on the growth and nutrient uptake of micropropagated oil palm
Menara Perkebunan **61**(3): 56–60

Winarsih S. 1996
Effect of phosphorus sources and mycorrhizal infection on growth of in vitro coffee plantlets
Pelita Perkebunana **12**(1): 16–24

Winarsih S, Baon J B, and Priyono. 1995
Studies on the use of bananas trees (Musa sp.) for coffee and cocoa shading. IV. Effect of phosphorus sources on VA mycorrhizal infection of micropropagated bananas
Pelita Perkebunan **11**(1): 56–63

Yang W Q and Goulart B L. 1997
Aluminium and phosphorus interactions in mycorrhizal and nonmycorrhizal highbush blueberry plantlets
Journal of the American Society for Horticultural Science **122**(1): 24–30

Yang W Q, Goulart B L, and Demchak K. 1996
The effect of aluminium and media on the growth of mycorrhizal and nonmycorrhizal highbush blueberry plantlets
Plant and Soil **183**(2): 301–308

Research findings

Comparative efficacy of different arbuscular mycorrhizal fungal species (AMF) on chickpea (*Cicer arietinum*)

Archana Gautam and *Irshad Mahmood*
Department of Botany, Aligarh Muslim University, Aligarh-202 002, India

Introduction

The importance of AM fungi in plant growth has now been fully appreciated and they have received considerable attention in the recent past owing to their beneficial effect on crop productivity (Hayman and Mosse 1977; Tilak 1993), increased resistance and tolerance to drought and root pathogens in many species of leguminous and other crop plants (Mosse 1973; Singh 1994). In general, efficient strains of fungi are those that infest and colonize the root system rapidly (Munns and Mosse 1980). Information on the selective association of AM fungi with the chickpea is meagre. In natural soils, chickpea is reported to be highly mycorrhizal (Jalali and Thareja 1981; Singh and Verma 1987).

In the present study, six VAM fungi viz. *Glomus fasciculatum, G. mosseae, G. constrictum, G. aggregatum, Acaulospora scrobiculata, Gigaspora gigantea* are evaluated individually for their potential as VAM inoculants. This study also provides evidence that among the leguminous crops, chickpeas are very efficiently colonized by *G. fasciculatum.*

Materials and methods

Chickpea variety Avrodhi was raised in sterilized clay pots (15 cm diameter) containing 950 g of sandy loam soil mixture (66% sand, 24% silt, 8% clay, 2% organic matter, pH 7.7). Before sowing the seeds, 50 g inoculum of different VAM fungi having 800 spores were added individually to the soil. Five replicates of each treatment were maintained.

Pots of all the treatments were kept in a glasshouse. Plants were uprooted after 90 days and the per cent colonization of mycorrhizal roots was recorded using the method of Phillips and Hayman (1970). Nodulation was also recorded in terms of the dry weight of nodules per plant. The spores were extracted from the root washings by the method of Gerdemann and Nicolson (1963) and the spore count of the rhizospheric soil recorded. The plant growth in terms of plant length, plant fresh weight, and plant dry weight was also recorded after the transplantation. Nitrogen, phosphorus and potassium were estimated by the method of Linder (1944) and Lundergardh (1951). All the data relating to the growth of plant, root infection and nutrient content were analysed statistically by the method of Panse and Sukhatme (1985) and the critical difference at the 5% level of significance was calculated.

Results

In general, the AM inoculates as evaluated for chickpea improved not only mycorrhizal colonization in

roots, but also nodulation, plant biomass and nutrient status (Table 1).

The plant dry weight remained the least in the control. Plants inoculated with *G. mosseae* had significantly higher dry weights than those inoculated with *G. aggregatum*. The highest total plant dry weight was supported by *G. mosseae* and *G. constrictum*. *Glomus fasciculatum* proved to be significantly superior to other species in increasing the N, P, and K content of the plants.

G. fasciculatum-inoculated plants showed the greatest nodulation followed by plants inoculated with *G. mosseae* and *G. constrictum*, while inoculation of *G. aggregatum* resulted in the same number of nodules as the control. The other two AM fungi, *G. gigantea* and *A. scrobiculata* caused a decrease in the number of nodules compared to the control.

Mycorrhization of plants treated with *G. fasciculatum* was higher compared to all other treatments. The mycorrhization in case of *A. scrobiculata* and *G. gigantea* was less than that of other AM fungi viz. *G. fasciculatum*, *G. mosseae,* and *G. constrictum*. The highest percentage of root colonization was observed in *G. fasciculatum* inoculated plants followed by *G. mosseae*.

Discussion

Glomus fasciculatum has been found more efficient overall, on chickpea compared to others, followed by *G. mosseae*. Ramraj and Shanmugan (1990) found *G. etunicatum* more effective in increasing the dry weight of the cowpea plant. Similar results have been obtained in chickpea by Singh and Verma (1987), where *G. fasciculatum* and *G. etunicatum* proved to be most effective. In the present study, *G. fasciculatum* was found to support the highest biomass production, mycorrhizal colonization, and nutrient uptake. It is therefore designated a potential AMF inoculant for the chickpea variety, Avrodhi.

Acknowledgement

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

References

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

Hayman D S and Mosse B. 1977
Plant growth responses to vesicular arbuscular mycorrhiza. I. Growth of Endogone inoculated plants in phosphate-deficient soils
The New Phytologist **70**: 19–27

Jalali B L and Thareja M L. 1981
Suppression of fusarium wilt of chickpea in vesicular arbuscular mycorrhiza-inoculated soils
International Chickpea Newsletter **4**: 21–22

Linder R C. 1944
Rapid analytical methods for some of the more common inorganic constituents of plant tissue
Plant Physiology **19**: 76–89

Lundegardh H. 1951
Leaf analysis pp. 65–66 (translated by R L Metchell)
London: Hilger and Hatts Limited

Mosse B. 1973
Advances in the study of vesicular arbuscular mycorrhiza
Annual Reviews of Phytopathology **17**: 171

Munns D N and Mosse B. 1980
Mineral nutrition of legume crops
In *Advances in Legume Science*, pp.115, edited by R J Summerfield and A H Bunting
England: University of Reading

Table 1 Effect of different arbuscular mycorrhizal fungi on the growth, nutrient status, nodulation and mycorrhization of chickpea var. Avrodhi

	Total plant			*Nutrient status (per cent)*				
Treatment	*Length (cm)*	*Fresh wt (g)*	*Dry wt (g)*	*N*	*P*	*K*	*Nodule number*	*Mycorrhization*
Control	65.86	53.46	8.23	2.63	0.248	1.90	15	–
Glomus fasciculatum	80.46	62.40	10.30	3.56	0.274	2.06	21	55
Glomus mosseae	76.50	60.96	10.00	3.00	0.259	2.02	18	52
Glomus constricutum	75.00	59.63	9.56	2.83	0.256	2.00	17	49
Glomus aggregatum	73.70	58.50	9.00	2.79	0.252	1.98	15	45
Gigaspora gigantea	71.50	56.50	8.40	2.66	0.250	1.93	10	42
Acaulospora scrobiculata	70.60	56.30	8.30	2.64	0.249	1.91	8	39
CD at 5%	8.73	3.80	0.39	0.05	0.005	0.05	2	4

Panse V G and Sukhatme P U. 1985
Statistical methods for agricultural works
New Delhi: Publications and Information Division, Indian Council of Agricultural Research, New Delhi. 355 pp.

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

Ramraj B and Shanmugan N. 1990
Effect of vesicular arbuscular mycorrhizal inoculation on cowpea: A field study, pp. 84–85
In *Mycorrhizal symbiosis and plant growth*, edited by D J Bagyaraj and A Manjunath
Bangalore: University of Agriculture Science, 132 p.
[Proceedings of the Second National Conference on Mycorrhiza, Bangalore, India, 21–23 November 1990]

Singh H P. 1994
Response to inoculation with *Bradyrhizobium*, vesicular arbuscular mycorrhiza and phosphate solubilizing microbes on soybean in a mollisol
Indian Journal of Microbiology **34**: 27–31

Singh K and Verma A K. 1987
Mycorrhizal fungi stimulate legume growth and root nodulation in dry and semi-arid soils. 1. Effect of dual inoculation of *Rhizobium* and VA mycorrhizal spores on a tropical legume bengal gram (*Cicer arietinum* L.)
In *Mycorrhiza Round Table*, pp. 356–371, edited by A K Varma
Ottawa: IDRC Publications

Tilak K V B R. 1993
Associative effects of vesicular arbuscular mycorrhiza with nitrogen fixers
Proceedings of the Indian National Science Academy **59**: 325–332

Occurrence of VAM (vesicular arbuscular mycorrhiza) in the roots of *Phyllanthus fraternus* Webster

R M Mulani, Rajendra R Prabhu, and *Manjusha Dinkaran*
Botany Research Laboratory, Department of Botany, Sheth L U Jhaveri College of Arts and Commerce and Sir M V College of Science, Dr Radhakrishnan Road, Andheri (East), Mumbai-400 069, India

Introduction

Phyllanthus fraternus (synonym *Phyllanthus niruri* L.) is an annual herbaceous, medicinal plant commonly occurring in marshy places and is supposed to be a weed. It is commonly called 'bhui awala' in Marathi, 'jangali ambli' in Hindi, and belongs to the family *Euphorbiaceae*. *P. fraternus* is found throughout tropical and subtropical regions of the world, and in India it is a common weed, found extensively from Kashmir to Kanyakumari. The plant has characteristic 'phyllanthoid' branching (Figure 1a).

The plant has medicinal value in the Ayurvedic system. The fresh plant extract mixed with cow's milk is given to patients suffering from jaundice. The herb is bitter in taste and is reported to possess astringent, diuretic, febrifugal and antiseptic properties. It is used in stomach troubles such as dyspepsia, colic, diarrhoea and dysentery. It is also useful in dropsy and diseases of the urinogenital system (Anonymous 1982).

The present study is part of a project on the occurrence of VAM in the soil of Mumbai and adjoining areas and deals with VAM colonization in the roots of *Phyllanthus fraternus*. Earlier reports indicated VAM colonization in a few genera of *Euphorbiaceae* (Mohan and Natrajan 1988; Raghupathy, Mohankumar, and Mahadevan 1988). Roots of *Phyllanthus niruri* are reported to contain little or no infection (Kanan and Lakshminarashiman, 1988).

Material and methods

The roots and rhizospheric soil of *Phyllanthus fraternus* were both collected from the college campus without damaging the root system. The root system of the plant along with rhizospheric soil were kept in a glass beaker of capacity 2 litre containing double distilled water for 3-4 hours to allow for the removal of the adhering rhizospheric soil and also for the separation of the roots. The roots were washed again with double distilled water in another beaker and then processed to check for the occurrence of VAM colonization using the modified process suggested by Phillips and Hayman (1970). Soil analysis was done using standard methods suggested by Jackson (1958).

The roots were first soaked in glycerine for 1 hour, followed by rinsing with double distilled water. They were then autoclaved at 121°C and 15 lb pressure for 20 minutes along with 1N KOH. On cooling, 3 to 5 drops of H_2O_2 followed by 1N HCl was added to neutralize the tissue and then it was stained with cotton blue, and checked for VAM colonization. The percentage of VAM colonization was determined using the formula suggested in the manual for identifying VAM fungi by Schenck and Perez (1988). The percentage of VAM colonization was calculated using the following formula:

$$\text{Percentage VAM colonization} = \frac{\text{Number of mycorrhizal root segments}}{\text{Total number of root segments}} \times 100$$

At the same time, 100 g of rhizospheric soil was processed to extract the resting live spores i.e. extramatricular chlamydospores, using the modified method suggested by Gerdemann and Nicolson (1963).

Rhizospheric soil (100 g) was dissolved in 1 litre of double distilled water containing a few drops of phosphate-lacking detergent. The solution was stirred well and allowed to stand still for 15 to 20 minutes. The rhizospheric solution was then sieved through a series of sieves with different pore sizes; 1 mm, 450 mm, 250 mm, 100 mm, 50 mm, and 25 mm, respectively. Since the spores were found only on the 100 mm and 50 mm sieves, their debris was collected and centrifuged in a 1% sugar solution at 100 rpm for 10–15 min. The supernatant was poured into a petri-dish containing a nylon mesh (20 mm pore size) for determining the percentage of spore distribution in the given quantity of soil.

Result and discussion

Table 1 gives the physico-chemical properties of rhizospheric soil and Table 2 gives VAM colonization in the roots of *P. fraternus*. The roots of *P. fraternus* were invariably mycorrhizal showing 75%–80% colonization. The secondary roots were mainly infected. The inter- and intracellular hyphae were coenocytic aseptate (Figure 1b, c). Intercellular hyphae forming intercellular vesicles and intracellular arbuscules were observed (Figure 1b).

The vesicles were observed both in the corticular and central stelar regions (Figure 1b). The vesicles were ovate, obovate, elongated, and irregularly-lobed with a large amount of stored food material in the form of oil globules (Figure 1d). The inter-corticular hyphae showed swellings within the host tissue, which may later lose their content to form dark, irregular bodies called arbuscules (Figure 1b) (Gallaund 1905). The mycorrhizal hyphae enter the host tissue by piercing epiblemal cells forming a peg-like structure called an appresorium (Figure 1b). The mycelium was observed running longitudinally throughout the root tissue (Figure 1b).

Physico-chemical analysis of rhizospheric soil showed that it was neutral, low in organic carbon content and nitrates, with a high water-holding capacity (Table 1). The average resting spore count in rhizosperic soil was 400 spores per 100 g of soil. Extramatricular hyphae as well as spores were observed in the soil. Most of the spores extracted from rhizospheric soil were of *Glomus* sp., while a few were of *Acaulospora* sp., *Gigaspora* sp. and sporocarp of *Sclorocystis* sp. The chlamydospores of *Glomus* sp. were in clusters or single, with straight and recurved hyphal attachments. The spores of *Glomus* sp. were thick-walled (Figure 2b, c) at the hyphal end (Figure 2d) whereas the wall of *Gigaspora* sp. spores were thin (Figure 2a). The spores of *Gigaspora* sp. had bulbous suspensors or sporogenous attachments.

Table 1 Physico-chemical properties of rhizospheric soil of *P. fraternus*

Characteristics		*Observations*
Soil texture (particle size analysis %)	i) Coarse sand	36.00%
	ii) Fine sand	52.70%
	iii) Silt	8.40%
	iv) Clay	2.90%
Colour		Brownish-black
Total soluble salts		0.798%
Bicarbonates		0.318%
Nitrates		0.007%
Moisture (at 110 °C)		7.75%
Water-holding capacity		40.80%
Percentage loss at 105°C		7.68%
pH		7.01
Organic carbon		2.52%
Chloride		0.809%

Table 2 Occurrence of VAM fungi in the roots and the percentage of resting spores in rhizospheric soil of ***P. fraternus***

Characteristic	*Observation*
VAM infection	+
Average percentage of VAM colonization	78%
Presence of intracellular hyphae	+
Presence of extramatricular hyphae	+
Presence of vesicles	+
Presence of intracellular arbuscules	+
Average number of spores per 100 g of soil sample	400
Type of mycorrhizal species	*i) Glomus sp.* *ii) Gigaspora sp.* *iii) Sclerocystis sp.* *iv) Acaulospora sp.*

VAM colonization was observed in various species of *Euphorbiaceae*. *Euphorbia rosea* was mycorrhizal for *Glomus pubescens*, *Glomus monosporous; Croton sparsiflorus* was mycorrhizal for *Glomus darus*; *Acalypha indica* for *Glomus pubiscence* (Mohan and Natrajan, 1988); *Euphorbia heterophylla* was mycorrhizal for *Glomus citricolum* (Raghupathy, Mohankumar, and Mahadevan 1988). VAM colonization was not observed in *Agyneria baccifermia* (Parmeswaran and Augustine, 1988); *Exoecaria agallocha* and *Croton passiflorus* (Kannan and Lakshminarshiman, 1988), *Exoecaria agallocha* and *Euphorbia hirta* L. (Ragupathy, Mohankumar, and Mahadevan 1988).

Earlier reports have also shown that species of *Phyllanthus* such as *P. madraspatensis* are non-

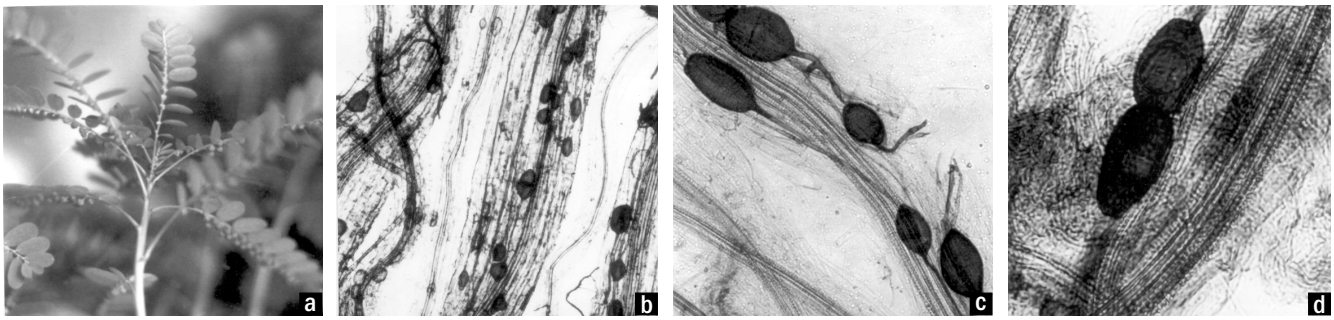

Figure 1 Occurrence of VAM fungi in the roots of ***Phyllanthus fraternus*** **Webster**
a) Twig of *Phyllanthus fraternus* with phyllanthoid branching x 1/10 ns.
b) Whole mount of VAM colonized root showing longitudinally running hyphae with vesicles in corticular and stellar region × 180
c) Lobed vesicles with coenocytic hyphae × 400
d) Vesicles containing oil globules × 600

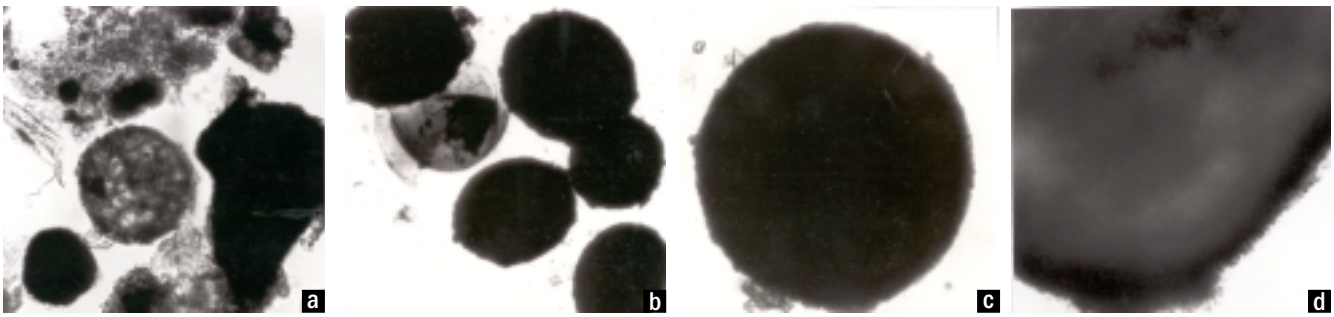

Figure 2 Occurrence of VAM fungi in the roots of ***Phyllanthus fraternus*** **Webster**
a) Chlamydospore of *Gigaspora sp*. with bulbous attachment x 400
b) Chlamydospore of *Gigaspora sp*. x 400
c) Chlamydospore of *Gigaspora sp*. x 600
d) Live chlamydospore of *Gigaspora sp*. of chocolate honey colour x 800

mycorrhizal (Mohan and Natrajan, 1988); *P. simplex* (Parmeswaran and Augustine, 1988) and even *P. niruri* are reported to be non-mycorrhizal while it was reported to be occasionally mycorrhizal by Kannan and Lakshminarashiman (1988). In the present investigation of *P. fraternus*, 78% mycorrhizal colonization of roots and 400 spores per 100 g of soil was observed. We therefore conclude that VAM colonization in the roots of *P. fraternus* is not hampered by the presence of secondary metabolites but may be due to some other edaphic factors.

Acknowledgements

The authors would like to thank DST, Maharashtra Government, for funding the present investigation, Professor A R Kulkarni for his valuable comments, and the Principal and management of the college for providing facilities.

References

Anonymous. 1982
Wealth of India raw material, *Volume VIII*
New Delhi: CSIR

Gallaund G. 1905.
On the study of endotrophic mycorrhizae
Rev. Gen. Bot **17**: 7–48

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

Jackson M L. 1958
Soil chemical analysis
London, UK: Constable

Kannan K and Lakshminarashiman C. 1988
Survey of VAM of maritime strand plants of Point Calimere, pp. 53–55
In *Mycorrhizae for Green Asia*, edited by A Mahadevan, N Raman, and K Natarajan
Madras: University of Madras. 351 pp.
[*Proceedings of the First Asian Conference on Mycorrhizae*, Madras, India, 29–31 January, 1988]

Mohan V and Natrajan K. 1988
VAM association in sand dunes plants in the Coromandel coast, pp. 73–76

In *Mycorrhizae for Green Asia*, edited by A Mahadevan, N Raman, and K Natarajan
Madras: University of Madras. 351 pp.
[*Proceedings of the First Asian Conference on Mycorrhizae*, Madras, India, 29–31 January, 1988]

Mohankumar V and Mahadevan A. 1988
Seasonal changes in spore density and root colonization of VAM in a tropical forest, pp. 77–79
In *Mycorrhizae for Green Asia*, edited by A Mahadevan, N Raman, and K Natarajan
Madras: University of Madras. 351 pp.
[*Proceedings of the First Asian Conference on Mycorrhizae*, Madras, India, 29–31 January, 1988]

Parameswaran P and Augustine B. 1988
Distribution and ecology of a VAM in a scrub jungle, pp. 91–94
In *Mycorrhizae for Green Asia*, edited by A Mahadevan, N Raman, and K Natarajan
Madras: University of Madras. 351 pp.
[*Proceedings of the First Asian Conference on Mycorrhizae*, Madras, India, 29–31 January, 1998]

Phillips J M and Hayman D S. 1970
Improved procedures 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

Raghupathy S, Mohankumar V, and Mahadevan A. 1988.
Distribution of VAM in Thanjavur district flora, pp. 96–98
In *Mycorrhizae for Green Asia*, edited by A Mahadevan, N Raman, and K Natarajan
Madras: University of Madras. 351 pp.
[*Proceedings of the First Asian Conference on Mycorrhizae*, Madras, India, 29–31 January, 1998]

Schenck NC and Perez Y. 1988
Manual for the identification of VA mycorrhizal fungi, pp. 19–36
USA: Synergistic Publications

Arbuscular mycorrhizal associations in *Tamarix aphylla* in the Indian Thar Desert

J Panwar and *A Vyas*
Department of Botany, JNV University, Jodhpur-342 001, Rajasthan, India

Introduction

Arbuscular mycorrhizal fungi occur in a wide range of plant habitats. Different species of AM fungi occur in arid and semi-arid regions in India (Mathur and Vyas 1997; Panwar and Vyas 2002). Marcel, Heijden, John et al. (1988) suggested the need to protect AM fungi and to consider them in future management practices in order to maintain diverse ecosystems. Their potential to enhance plant growth and biomass is well known. Thus, AM fungi are also known as biofertilizers.

Tamarix aphylla is an important multipurpose endangered tree species in the Thar Desert. It is a source of fuel, fodder and timber. Besides the nature of the host plant, environmental conditions have a significant effect on the distribution, density and composition of arbuscular mycorrhizae (Daniels and Trappe 1980). The present investigation was undertaken to study the distribution of AM fungi in this endangered tree species.

Material and methods

A survey of five localities, Jaisalmer, Barmer, Bikaner, Nagaur, and Osian in the Thar Desert was undertaken periodically to collect rhizosphere soils along with roots of *T. aphylla*. The collections were made throughout the year. Soil samples collected were processed to obtain spores (Gerdemann and Nicolson 1963). The identification of AM species was made using the manual of Schenck and Perez (1987) and Morton (1988). The root samples collected from different localities were gently washed under tap water and stained using the method of Phillips and Hayman (1970) to calculate the percentage of root colonization (Giovannetti and Mosse 1980). Physico-chemical characteristics of soil samples such as pH, soil moisture and soil texture (Subbiah and Asija 1956) were estimated. Soil organic carbon was estimated by the method of Walkey and Black (1934) and soil phosphorous by the method of Olsen, Watanable, and Danielson (1963). Nitrogen in soil samples was estimated by the Kjeldhal method (Bremner 1960).

Results and discussion

The soil of the Indian Thar Desert is highly sandy. Its pH varied from 7.4–10.2 and was alkaline by nature. The organic carbon content varied from 0.40–0.50%. Phosphorous and nitrogen varied from 18–35 kg/hectare and 20–38 kg/hectare, respectively. Soil moisture at different localities varied from 8.10%–9.15% (Table 1). AM fungal spore population and percentage root colonization are presented in Table 2. The mycorrhizal spore population in different localities varied from 120–225 spores/g of soil, while the percentage of root colonization varied from 45–60%.

Spores of ten AM fungal species belonging to five genera were isolated from the rhizosphere of *T. aphylla* at different localities (Table 3). One species of *Acaulospora*, two species of *Gigaspora*, four species of *Glomus*, one species of *Sclerocystis,* and two species of *Scutellospora* were isolated and identified.

Table 1 Physico-chemical characteristics of rhizosphere soils of *T. aphylla* at different localities

Collection site	*Coarse sand (%)*	*Fine sand (%)*	*Silt (%)*	*Clay (%)*	*Soil pH*	*Soil moisture (%)*	*Organic carbon (%)*	*Soil P kg/hectare*	*Soil N kg/hectare*
Jaisalmer	2.5	89.5	3.7	4.3	10.2	9.12	0.45	18	20
Barmer	2.8	89.2	3.5	4.5	7.4	8.10	0.40	35	38
Bikaner	3.3	86.6	3.8	6.3	8.3	9.15	0.43	30	34
Nagaur	5.5	87.4	1.8	5.3	9.4	8.45	0.50	22	25
Osian	5.8	88.0	1.7	4.5	9.0	8.80	0.45	25	28

Table 2 Percentage of root colonization and AM spore population in rhizosphere of *T. aphylla* at different localities

Locality	*Percentage root colonization*	*AM spores per g soil*
Jaisalmer	60	225
Barmer	45	120
Bikaner	48	140
Nagaur	55	190
Osian	52	162

Table 3 Distribution of arbuscular mycorrizal fungi in the rhizosphere of *T. aphylla* at various localities

	Locality				
AM fungi	*1*	*2*	*3*	*4*	*5*
Acaulospora mellea	+	+	+	+	+
Gigaspora gigantea	++	++	++	++	++
Gigaspora margarita	++	++	++	++	++
Glomus aggregatum	–	+	–	–	–
G. constrictum	+	++	++	++	+
G. deserticola	+++	+++	+++	+++	+++
G. fasciculatum	++	++	++	++	++
Sclerocystes rubiformis	+	+	–	–	+
Scutellaspora calospora	+++	+++	+++	+++	+++
Scutellaspora nigra	+	+	+	+	++

Locality 1= Jaisalmer, 2=Barmer, 3=Bikaner, 4=Nagaur, 5=Osian
Absent = –, Low = +, Moderate = ++, High = +++

The population of AM fungi at different localities was determined in terms of resting spores and sporocarps in the soil. The AM fungal endophytes were widespread in all the soils investigated but varied both in number and type of spores and sporocarps. The mycorrhizal spore population and percentage of root colonization were found to be affected by abiotic factors. Increase in soil phosphorus and nitrogen was correlated with a decrease in spore population and root colonization by AM fungi. Similarly, increase in soil pH was correlated with an increase in spore population and root colonization.

The present study reveals a definite AM fungal association in *T. aphylla*. The study also reveals a qualitative difference in the occurrence of different AM species, which is probably due to abiotic factors (Rose 1988). Blaszkowski (1993) while investigating plant communities in Poland, observed a significant correlation between spore density and soil pH. Mathur and Vyas (1997) also reported similar results. It is a well known fact that nutritionally deficient soils, particularly those deficient in phosphorus, harbour more AMF (Manoharachary, Vani, Satya Prasad, et al. 1996). The present study also confirms this observation.

No AM fungi have been reported so far from rhizospheres soil of *T. aphylla*. The present study provides an interesting and original account of AM fungi species associated with *T. aphylla* indicating the mycorrhizal dependency of the plant. Artificial inoculation of one or some of these indigenous AM fungi may prove useful in the re-establishment of this multipurpose endangered plant species in the Indian Thar Desert.

References

Blaszkowski J. 1993
The occurrence of arbuscular fungi and mycorrhizae Glomales in plant communities of maritime dunes and shores of Poland
Bulletin of the Polish Academy of Sciences, Biological Sciences **41**: 377–392

Bremner J M. 1960
Determination of nitrogen in soil by Kjeldahl method
Journal of Agricultural Science **55**: 11–33

Daniels BA and Trappe J M. 1980
Factors affecting spore germination of the vesicular arbuscular mycorrhizal fungus *Glomus epigaeus*
Mycologia **72**: 475–483

Gerdemann J W and Nicolson 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

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

Manoharachary C, Vani P, Satya Prasad K, and Prakash P. 1996
Vesicular-arbuscular mycorrhizal symbiosis with special reference to castor production
Perspectives of Biological Science 197–216

Marcel G A, Heijden V, John N K, Margot , Moutoglis P, Streilwolf Engel, Boller T, Weinken A, and Sanders I R. 1998
Mycorrhizal fungal diversity determines plant biodiversity ecosystem variability and productivity
Nature **396**: 69–72

Mathur N and Vyas A. 1997
Vesicular arbuscular mycorrhizae in Indian Thar desert
Journal of the Indian Botanical Society **76**: 125–126

Morton J B. 1998
Taxonomy of VA mycorrhizal fungi: Classification, nomenclature and identification
Mycotaxon **32**: 267–324

Olsen S R, Watanable P S, and Danielson R E. 1963
Phosphorus absorption by corn roots as affected by moisture and phosphorus concentration
Soil Science Society of America Journal **25**: 289–294

Panwar J and Vyas A. 2002
Occurrence of arbuscular mycorrhizal fungi in rhizosphere of an endangered tree species of Indian Thar desert
Biofertilizer Newsletter **8**(2): 14–16

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

Rose S L. 1988
Above and below ground community development in marine sand dune ecosystem
Plant and Soil **109**: 215–226

Schenck N C and Perez V. 1987
Manual of identification of VA mycorrhizal fungi
Florida: Fifield Hall University. 241 pp.

Subbiah B V and Asija G L. 1956
A rapid procedure for determination of available nitrogen in soils
Current Science **25**: 259–260

Walkey A and Black T A. 1934
An examination of the Degtiareff method for determination soil organic matter and proposed modification of the chromic acid titration
Soil Science **37**: 29–38

VAM and better growth of micropropagated banana

M N Thaker and *Y T Jasrai**
Department of Botany, Faculty of Science, M S University of Baroda Vadodara-390 002, India

Introduction

Vesicular-arbuscular mycorrhiza (VAM) are a distinct group of fungi that form symbiotic relationships with the roots of higher plants. The role of VAM in increasing the uptake, drought tolerance, reducing pathogen invasion, transplant mortality, and improving water relations (Adholeya and Gaur, 1999) in various ornamentals and horticultural plants has been documented.

Banana, a the horticultural crop, is extensively cultivated in India (0.34 m hectares) with an annual yield of 6.2 m tonnes at the rate of 18 tonnes per hectare (Upadhyaya, 1995). In recent years, a considerable portion of this yield has been achieved through micropropagation. These micropropagated plants, being generated in vitro, lack any symbiotic association indigenously. In order to enhance the growth efficiency of micropropagated banana, an ecofriendly approach involving the association of VAM needs to be explored.

In the present study, the effect of VAM on various growth parameters and the chlorophyll

* author for correspondence: e-mail- yjasrai@yahoo.com

content of micropropagated banana (*Musa paradisiaca* var. *robusta*) were studied during early growth.

Methods and materials

Micropropagated banana plantlets of equal size and weight were selected.

The plantlets were individually planted in polybags (1 litre capacity) containing 1.25 kg/bag sterilized soil mixture (soil:sand:compost; 2:1:1) and served as the control (non-mycorrhizal/without VAM). There were 30 plantlets maintained per set. A set of plantlets was inoculated with 5 g of soil containing VAM consortia (215 spores/g of soil) and treated as mycorrhizal/VAM-inoculated plants. The two sets of plantlets were grown in the botanical garden of M S University of Baroda between June and September.

Quantification of growth

After 90 days of growth, plantlets from both the sets were uprooted carefully without injuring the root system. The rhizospheric soil adhering to roots was removed under tap water and various growth parameters such as leaf size (breadth, length, area), girth of pseudostem, fresh weight of shoot and root were recorded.

Quantification of chlorophyll

The leaf chlorophyll was extracted in dimethyl sulphoxide (Barnes and Balaguer, 1992) and quantified essentially as reported earlier (Maclachalan and Zalik, 1963) using a uv-visible spectrophotometer (Shimadzu).

Staining procedure

The isolated banana roots were stained with acid fuchsin (Bhagyaraj, Shashikala, and Reddy 1999).

Percentage colonization was calculated following Nicholson's formula:

$$\text{Percentage root colonization} = \frac{\text{Number of segments with VAM}}{\text{Total number of segments}} \times 100$$

Results and discussion

The results presented in Table 1 reveal a highly significant effect of VAM in enhancing the growth of micropropagated banana at the end of 90 days. The shoot length of the mycorrhizal plantlets increased by more than 82.34% accompanied by a corresponding increase in shoot weight (85.74%), over the non-mycorrhizal plants. The roots showed a significant increase of 114.59 % in root weight with only 17.14% increase in root length of the VAM treated plants compared to the control.

The major site of photosynthesis, the leaves, also increased in length, breadth, and area compared to the control plantlets by 52.9%, 63.11%, and 158.63%, respectively. The girth of the pseudostem measured at the base was also more by 48.71% in mycorrhizal plants than in non-mycorrhizal ones. The enhancement in the growth of micropropagated banana plantlets can be attributed to the higher content of chlorophyll (42.35%) in VAM-infected plants in contrast to the control.

Table 1 Effect of VAM colonization on various growth parameters in micropropagated banana

Growth parameter	*Treatment*	*Growth Initial*	*After 90 days ± S.E.*
Shoot length (cm)	Control	4.00	5.55 ± 0.297
	Control + VAM		10.12 ± 0.507
Root length (cm)	Control	2.59	2.89 ± 0.600
	Control + VAM		3.40 ± 0.550
Shoot weight (g)	Control	1.67	4.93 ± 0.348
	Control + VAM		9.16 ± 0.376
Root weight (g)	Control	0.69	1.37 ± 0.132
	Control + VAM		2.94 ± 0.121
Stem girth (cm)	Control	1.60	1.95 ± 0.067
	Control + VAM		2.90 ± 0.095
Leaf length (cm)	Control	6.00	9.20 ± 0.472
	Control + VAM		14.07 ± 0.469
Leaf breadth (cm)	Control	2.80	3.40 ± 0.166
	Control + VAM		5.75 ± 0.256
Leaf area (cm^2)	Control	16.80	31.28 ± 0.637
	Control + VAM		80.90 ± 0.725
Chlorophyll content (mg/g fresh wt)	Control	1.41	1.46 1.102
	Control + VAM		2.07 1.324

Initially the trypan blue technique (Phillips and Hayman, 1970) was employed for staining VAM in roots but clear results were not obtained. This could be because banana roots are highly pigmented and need effective bleaching. In order to detect and evaluate VAM colonization, the roots were stained by the modified method of Bhagyaraj, Shashikala, and Reddy (1999). The high percentage of root colonization (87%) in the treated plants could have resulted in better nutrient uptake, thus increasing the chlorophyll levels, and thereby, root and shoot growth.

These significant results indicate the potential of VAM as an aid to increasing the vigour of micropropagated plants of an economically valuable crop such as the banana.

References

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

Barnes J D and Balaguer L. 1992
A reappraisal and use of DMSO for extraction and determination of chlorophyll a and chlorophyll b in lichens and higher plants
Environmental and Experimental Botany **32**: 85–100

Bhagyaraj D J, Shashikala B N, and Reddy B J D. 1999
Response of micropropagated banana plantlets to *Glomus mosseae* at varied levels of fertilizer phosphorous
Journal of Experimental Botany **37**: 499–502

Maclachalan S and Zalik S. 1963
Plastid structure and free aminoacid composition of a chlorophyll mutant of barley
Canadian Journal of Botany **41**: 1053–1062

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**: 158–160

Upadhayaya D C. 1995
Drip irrigation in banana has an edge over flow irrigation
Indian Horticulture **45**(2): 13–15

New approaches

In situ extraction of rhizosphere organic compounds

A method is described by Morse C C, Yevdokimov I V, DeLuca T H (2000) for in situ extraction of rhizosphere organic compounds (Communications in Soil Science and Plant Analysis 31(5–6): 725–742). Polyester capsules containing the non-ionic carbonaceous resins, Ambersorb 563 or XAD-7 were placed within the rhizosphere of spotted knapweed (*Centaurea maculosa*) and the native grass, Idaho fescue (*Festuca idahoensis*), as well as a bare-soil control in both greenhouse and field conditions. At the end of a 14-day period, resins were removed and extracted using sequential elution with water, 50% methanol, and 100% methanol. The eluent fractions were then analyzed for total organic carbon, total hexose sugars as anthrone reactive carbon, and total phenols. Samples were then concentrated by rotary evaporation to dryness and analyzed using HPLC with a C-18 column and tunable ultraviolet detector or a photodiode array detector. Ambersorb 563 resin extracts in the rhizosphere of knapweed from greenhouse and field trials always had twice as much soluble C, and 3–7 times more total soluble sugars compared to Idaho fescue during the 1996 and 1997 field seasons. This difference was not observed using the XAD-7 resins during the rather wet season of 1998. In these studies, fescue was found to release higher levels of sugars than knapweed, but these levels were not significantly different from those in the control soils. Compounds sorbed to the resins from the knapweed rhizosphere were more effectively eluted by methanol than water and demonstrated both the presence of carbohydrate groups and UV absorption. The XAD-7 resins allow for sorption of phenolic compounds as Ambersorb 563 does but allow for far greater desorption of these compounds. Non-organic resins may provide an effective means of capturing rhizosphere organic compounds in situ, but the low concentrations of sorbed compounds may limit their utility.

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.

- *Applied Biochemistry and Biotechnology*
- *Current Science*
- *FEMS Microbiology Ecology*
- *Forest Ecology and Management*
- *Journal of Ecology*
- *Journal of Environmental Quality*
- *Journal of Experimental Botany*
- *Microbiological Research*
- *Mycotaxon*
- *The New Phytologist*
- *Plant and Soil*
- *Plant Molecular Biology*
- *Restoration Ecology*
- *Scientia Horticulturae*
- *Tree Physiology*

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

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos [*address of the corresponding author]
DeAraujo A A* and Roussos S	**A technique for mycelial development of ectomycorrhizal fungi on agar media** *Applied Biochemistry and Biotechnology* **98**: 311–318 [*De Araujo AA, UNICAMP (Universidade de Campinas), Food Engineering School, Bioaroma Lab, CNPq, CP 6121, BR-13083970 Campinas, SP, Brazil]
Muthukumar T* and Udaiyan K. 2002	**Arbuscular mycorrhizal fungal composition in semi-arid soils of Western Ghats, southern India** *Current Science* **82**(6): 624–628 [*Muthukumar T, Bharathiar University, Department of Botany, Microbiological Lab, Coimbatore 641046, Tamil Nadu, India]
Hagerberg D* and Wallander H. 2002	**The impact of forest residue removal and wood ash amendment on the growth of the ectomycorrhizal external mycelium** *FEMS Microbiology Ecology* **39**(2): 139–146 [*Hagerberg D, Lund University, Department of Microbial Ecology, Ecology Building, SE-22362 Lund, Sweden]
Wallander H*, Johansson L, and Pallon J. 2002	**PIXE analysis to estimate the elemental composition of ectomycorrhizal rhizomorphs grown in contact with different minerals in forest soil** *FEMS Microbiology Ecology* **39**(2): 147–156 [*Wallander H, Lund University, Department of Microbial Ecology, Ecology Building, S-22362 Lund, Sweden]
Huat O K*, Awang K, Hashim A, and Majid N M. 2002	**Effects of fertilizers and vesicular-arbuscular mycorrhizas on the growth and photosynthesis of *Azadirachta excelsa* (Jack) Jacobs seedlings** *Forest Ecology and Management* **158**(1–3): 51–58 [*Huat O K, University Putra Malaysia, Faculty of Forestry, Serdang 43400, Selangor, Malaysia]
Pampolina N M, Dell B*, and Malajczuk N. 2002	**Dynamics of ectomycorrhizal fungi in an *Eucalyptus globulus* plantation: effect of phosphorus fertilization** *Forest Ecology and Management* **158**(1–3): 291–304 [*Dell B, Murdoch University, School of Biological Science and Biotechnology, Murdoch, WA 6150, Australia]
Ba A M*, Sanon K B, and Duponnois R. 2002	**Influence of ectomycorrhizal inoculation on *Afzelia quanzensis* Welw. seedlings in a nutrient-deficient soil** *Forest Ecology and Management* **161**(1-3): 215–219 [*Ba A M, University Antilles Guyane, Laboratoire Biologie and Physiologie Vegetales, Faculty of Science, BP 592, F-97159 Pointe a Pitre, Guadeloupe]

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]
Helgason T, Merryweather J W, Denison J, Wilson P, Young J P W, and Fitter A H*. 2002	**Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland** *Journal of Ecology* **90**(2): 371–384 [*Fitter A H, University of York, Department of Biology, POB 373, York YO10 5YW, N Yorkshire, England]
Kosuta S, Hamel C*, Dalpe Y, and StArnaud M. 2002	**Copper release from chemical root-control baskets in hardwood tree production** *Journal of Environmental Quality* **31**(3): 910–916 [*Hamel C, McGill University, Department of Natural Resource Science, Macdonald Campus, 21 111 Lakeshore Road, St Anne De Bellevue, PQ H9X 3V9, Canada]
RiveraBecerril F, Calantzis C, Turnau K, Caussanel J P, Belimov A A, Gianinazzi S, Strasser R J, and Gianinazzi-Pearson V*. 2002	**Cadmium accumulation and buffering of cadmium-induced stress by arbuscular mycorrhiza in three *Pisum sativum* L. genotypes** *Journal of Experimental Botany* **53**(371): 1177–1185 [*Gianinazzi-Pearson V, University of Bourgogne, INRA, UMR 1088, BBCE, IPM, CMSE, BP 86510, F-21065 Dijon, France]
Hartwig U A*, Wittmann P, Raun R B, HartwigRaz B, Jansa J, Mozafar A, Luscher A, Leuchtmann A, Frossard E, and Nosberger J. 2002	**Arbuscular mycorrhiza infection enhances the growth response of *Lolium perenne* to elevated atmospheric pCO(2)** *Journal of Experimental Botany* **53**(371): 1207–1213 [*Hartwig UA, University of Hohenheim, Institute of Crop and Grassland Science 340, D-70593 Stuttgart, Germany]
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]
Blaszkowski J*, Adamska L, and Madej T. 2002	***Glomus lamellosum* (Glomales, Zygomycotina), an arbuscular mycorrhizal fungal species new for Poland and Europe** *Mycotaxon* **81**: 281–291 [*Blaszkowski J, University of Agriculture, Department of Plant Pathology, Slowachiego 17, PL-71434 Szczecin, Poland]
Gardes M*. 2002	**An orchid-fungus marriage – physical promiscuity, conflict and cheating** *The New Phytologist* 154(1): 4–7 [Gardes M*, University Toulouse 3, CESAC, CNRS, 29 Rue Jeanne Marvig, F-31055 Toulouse, France]
O'Connor P J*, Smith S E, and Smith E A. 2002	**Arbuscular mycorrhizas influence plant diversity and community structure in a semiarid herbland** *The New Phytologist* **154**(1): 209–18 [* O'Connor P J, University Adelaide, Department of Soil and Water, Waite Campus, PMB 1, Glen Osmond, SA 5064, Australia]
Lilleskov E A*, Hobbie E A, and Fahey T J. 2002	**Ectomycorrhizal fungal taxa differing in response to nitrogen deposition also differ in pure culture organic nitrogen use and natural abundance of nitrogen isotopes** *The New Phytologist* **154**(1): 219–231 [*Lilleskov EA, University of California, Berkeley, Department of Plant Microbial Biology, 111 Koshland Hall, Berkeley, CA 94720 USA]
McKendrick S L, Leake J R*, Taylor D L, and Read D J. 2002	**Symbiotic germination and development of the myco-heterotrophic orchid *Neottia nidus-avis* in nature and its requirement for locally distributed *Sebacina* spp.** *The New Phytologist* **154**(1): 233–247 [*Leake J R, University of Sheffield, Department of Animal and Plant Science, Sheffield S10 2TN, South Yorkshire, England]
Poulton J L, Bryla D, Koide R T*, and Stephenson A G. 2002	**Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato** *The New Phytologist* **154**(1): 255–264

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]
	[*Koide RT, Penn State University, Department of Horticulture, 102 Tyson Building, University Park, PA 16802 USA]
Brundrett M C*. 2002	**Coevolution of roots and mycorrhizas of land plants** *The New Phytologist* **154**(2): 275–304 [*Brundrett M C, Botanical Gardens and Park Author, Kings Park and Botanical Garden, Perth, WA 6005, Australia]
Cairney J W G* and Ashford A E. 2002	**Biology of mycorrhizal associations of epacrids (Ericaceae)** *The New Phytologist* **154**(2): 305–326 [*Cairney JWG, University Western Sydney, Centre Horticulture and Plant Science, Mycorrhiza Research Group, Parramatta Campus, Locked Bag 1797, Penrith S DCL, NSW 1797, Australia]
ShaulKeinan O, Gadkar V, Ginzberg I, Grunzweig J M, Chet I, Elad Y, Wininger S, Belausov E, Eshed Y, Arzmon N, BenTal Y, and Kapulnik Y*. 2002	**Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices*** *The New Phytologist* **154**(2): 501–507 [*Kapulnik Y, Agriculture Research Organisation, Volcani Ctr, IL-50250 Bet Dagan, Israel]
Gobert A and Plassard C*. 2002	**Differential NO_3- dependent patterns of NO_3- uptake in *Pinus pinaster*, *Rhizopogon roseolus* and their ectomycorrhizal association** *The New Phytologist* **154**(2): 509–516 [*Plassard C, INRA (Institut National de la Recherché Agronomique), AgroM, Equipe Rhizosphere and Symbioses, UMR 388, 2 Pl Viala, Batiment 13, F-34060 Montpellier 01, France]
Balasubramanian S, Kim S J, and Podila G K*. 2002	**Differential expression of a malate synthase gene during the preinfection stage of symbiosis in the ectomycorrhizal fungus *Laccaria bicolor*** *The New Phytologist* **154**(2): 517–527 [*Podila G K, Michigan Technology University, Department of Biology Science, Houghton, MI 49931 USA]
Zhou Z H*and Hogetsu T. 2002	**Subterranean community structure of ectomycorrhizal fungi under *Suillus grevillei* sporocarps in a *Larix kaempferi* forest** *The New Phytologist* **154**(2): 529–539 [*Zhou Z H, University Tokyo, Asian National Environmental Science Centre, Symbiotic Function Research Unit, Midori Cho 1-1-8, Nishitokyo, Tokyo 1880002, Japan]
Domisch T*, Finer L, Lehto T, and Smolander A. 2002	**Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings** *Plant and Soil* **239**(2): 173–185 [*Domisch T, Finnish Forest Research Institute, Joensur Research Centre, POB 68, F-80101 Joensuu, Finland]
Wu T H*, Hao W Y, Lin X G, and Shi Y Q. 2002	**Screening of arbuscular mycorrhizal fungi for the revegetation of eroded red soils in subtropical China** *Plant and Soil* **239**(2): 225–235 [*Wu T H, Penn State University, Department of Horticulture, 103 Tyson Building, University Park, PA 16802 USA]
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

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]
	[*Moynahan OS, University of Montana, Division of Biological Science, Missoula, MT 59812 USA]
Stahl P D*, Perryman B L, Sharmasarkar S, and Munn LC. 2002	**Topsoil stockpiling versus exposure to traffic: A case study on in situ uranium wellfields** *Restoration Ecology* **10**(1): 129–137 [*Stahl PD, University of Wyoming, Department of Renewable Resources, Laramie, WY 82071 USA]
Declerck S*, Risede J M, and Delvaux B. 2002	**Greenhouse response of micropropagated bananas inoculated with in vitro monoxenically produced arbuscular mycorrhizal fungi** *Scientia Horticulturae* **93**(3–4): 301–309 [*Declerck S, University Catholique Louvain, Unite Microbiologie, 3 Pl Croix Sud, B-1348 Louvain, Belgium]
Niemi K*, Haggman H, and Sarjala T. 2002	**Effects of exogenous diamines on the interaction between ectomycorrhizal fungi and adventitious root formation in Scots pine in vitro** *Tree Physiology* **22**(6): 373–381 [*Niemi K, University Kuopio, Department of Ecology and Environmental Science, POB 1627, FIN-70211 Kuopio, Finland]

Centre for Mycorrhizal Culture Collection

Mycorrhizal technology in fly ash reclamation

Centre for Mycorrhizal Research, Tata Energy Research Institute, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi – 110 003, India

Fly ash, which contains silica, aluminium, oxides of iron, calcium, magnesium, arsenic, mercury, cadmium, and other toxic metals, is a by-product of coal-fired power plants. Fly ash is a serious source of air pollution since it remains airborne for a long period and causes health hazards. There are about 82 utility thermal power stations, in addition to several captive power plants, using bituminous coal (with ash content > 30%) that produce approximately 85 million tonnes of fly ash per annum. Such a huge quantity poses challenges in the form of land usage, health hazards, and environmental dangers.

The use/disposal of ash is an environmentally safe manner is a critical issue in India. The problem of fly ash is tackled to some extent, by using it as a supplementary cementitious material for concrete and concrete products owing to its excellent pozzolanic properties. Its geotechnical properties permit it to be used in soil stabilization and as a fine filler in asphalt and other products. Because its physico-chemical properties are similar to those of soil, and essential plant nutrients are available, it can also be used for agricultural/soil amendments to some extent. In India, about 26 300 hectares of land are laid waste by fly ash dumps, and ways of utilizing it must be found which do not compromise on health and environmental safety.

The potential of mycorrhizal fungi in the reclamation of wastelands is well known, if not well exploited. Wastelands are drastically disturbed lands where native vegetation and animal communities have been removed and the topsoil has been lost, altered or buried. Wastelands may be created where minerals (coal, paoline, bauxite, gravel, sand, etc.) have been mined, or where land has been used to dump mining wastes. Land from which top soil has been removed for use elsewhere, may create 'borrow pits', saline soils, acidic soils, calcareous soils, wind- and water-eroded sites. The natural rehabilitation of such lands is a gradual process.

The Centre for Mycorrhizal Research has developed mycorrhizal technology, which incorporates the production and application of mycorrhizal biofertilizers to agricultural, horticultural, and silvicultural plant species. This technology has been developed in an effort to expedite rehabilitation using mycorrhizal biofertilizers.

The technology is attractive, as it requires inputs smaller than conventional ones, in maintaining the ash dykes and also results in the greening of fly ash

ponds without amending good earth from elsewhere, and without the use of inorganic fertilizers. This technology immobilizes heavy metals checking ground water contamination, checks fugitive emissions, and allows common, but important plant species to grow on the ash surface in a sustainable manner, adding to green cover.

This technology was used to reclaim fly ash dumps at the Badarpur Thermal Power Station (3900 m^2) and the Korba Thermal Power Station (33 760 m^2). A variety of commercially viable plants, including tree species, floriculture and aromatic species were established and were able to grow on fly ash overburdens following the application of this technology. The tree species used for reclamation have economic and commercial value, and are long-tern sources of revenue. Species established and grown may be sources of timber (*Shorea robusta, Tectona grandis),* fodder (*Albizzia procera, Casuarina equisetifolia, Melia azadirach),* tannin (*Acacia nilotica*), paper and pulp (*Dendrocalamus strictus*, *Populus euphratica, Eucalyptus tereticornis. Gmelina arborea* which is used for making musical instruments was also cultivated. Commercially and medicinally important herbs such as *Mentha arvensis* and *Vetiver zizanoides*, which produce commercially important oils, have been successfully planted. Ornamental plants such as *Polianthus tuberosa, Helianthus,* and *Tagetus erecta,* nitrogen fixers like *Sesbania aculeata,* and species good for soil aggregation such as aloe gel, *Agave sisalana* have been established and are in very good health.

Once vegetative cover has been established, very little maintenance is required. Fugitive dust emissions also come down after a while, once the ash surface is reclaimed. Otherwise, to prevent fly ash from being airborne, the dumping grounds need to be kept wet all the time, for which either sprinklers are used or agencies hired to water the grounds. Thermal power plants in India reportedly spend 7 billion rupees annually on this activity. Most dumping sites of this kind in India are not lined resulting in seepage, which contaminates the groundwater and soil. Reclamation activities not only improve the performance and growth characteristics of a species but also provide better commercial options for these areas. Many plants have the ability to absorb pollutants, particularly heavy metals, and can therefore be used to prevent a potential threat to ground water quality.

A significant restoration in soil quality with respect to the percentage of nitrogen, available phosphorus, available potassium, organic carbon, dehydrogenase activity and total microbial population was recorded after two years of plantation at both sites. Porosity and water-holding capacity increased significantly, and heavy metal toxicity in fly ash decreased. The heavy metal content in the leachate also decreased drastically as a result of reclamation activities. The heights of various tree species showed tremendous improvement during the growth period.

The time required for ash reclamation by this technology is the least but the method is more permanent than any other. The pilot model demonstration conducted by TERI has conclusively proved that using mycorrhiza can successfully achieve the greening of fly ash overburdens, without using good earth or chemicals. The advantages of such a demonstration are manifold: capacity building with the NTPC, multiplier effect for other thermal power stations within and outside the premises of the NTPC, employment generation for the affected community, increased area under ground cover, and most importantly, addressing the environmental concerns of ash dump management.

With land becoming scarce and ash production increasing phenomenally, there is an urgent need to address this problem earnestly. The fact that the reclamation of ash ponds enhances vegetative cover and several other benefits, should encourage serious attempts in this direction. Ash utilization has been at a dismal 3%–4% in the country despite serious efforts, so the reclamation of ash ponds should be viewed as an important option for ash utilization and also regaining improved landform.

Acknowledgements

The work done for the above received partial financial support from the Fly Ash Mission – Technology Information Forecasting and Assessment Council, New Delhi; National Thermal Power Corporation, NOIDA; Korba Super Thermal Power Station, Korba (Chhatisgarh); and the Indo-French Centre for the Promotion of Advanced Research, New Delhi, which is duly acknowledged.

References

Adriano D C, Page A L, Elseewi A A, Chang A C, and Straugham I A. 1980
Utilization and disposal of fly ash and other coal residues in terrestrial ecosystems (A review)
Journal of Environmental Quality **9**: 333–344

Carlson C L and Adriano D C. 1993
Environmental impacts of coal combustion on residues
Journal of Environmental Quality **22**: 227–247

Jastrow J D, Zimmerman C A, Dvorak A J, and Hinchman R R. 1981
Plant growth and trace element uptake on acid coal refuse amended with lime or fly ash
Journal of Environmental Quality **10**: 154–160

Petruzzelli G, Lubrano L, and Cervelli S. 1987
Heavy metal uptake by wheat seedlings grown in fly ash-amended soils
Water Air and Soil Pollution **32**: 389–395

Salter P J, Webb D S, and Williams J B. 1971
Effects of pulverized fuel ash on the moisture characteristics of coarse textured soils and on crop yields
Journal of Agricultural Science **77**: 56–60

Schwab A P, Tomecek M P, and Ohlenbusch P D. 1991
Plant availability of lead, cadmium, and boron in amended coal ash
Water, Air and Soil Pollution **57/58**: 297–306

Forthcoming events

Conferences, congresses, seminars, symposiums, and workshops

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

International Conference on Environmentally Sustainable Agriculture for Dry Areas for the 2nd Millennium
Mrs Catherine Vachon, Lethbridge Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta Canada T1J 4B1

Tel. 1 403 317 2257 • *Fax* 1 403 382 3156
E-mail vachonc@em.agr.ca
Web site http://res2.agr.ca/lethbridge/hebei/confindex.htm

Beijing, **China**
16–20 September 2002

International Rice Congress 2002

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

Cairo, **Egypt**
26–28 October 2002

Agro Environ 2002 Sustainable Agro-Environmental Systems
Prof. Sami Abdel-Rehman, Symposium Secretary General, National Authority for Remote Sensing and Space Sciences, 23 Joseph Broz Tito St., El Nozha El Gedida, P.O. Box 1564 Alf-Maskan, Cairo, Egypt

Tel. 202 296 4386, 297 5688 • *Fax* 202 296 4387, 296 4385
E-mail sirahman@intouch.com

Urbana, Illinois, **USA**
7–8 January 2003

Illinois Crop Protection Technology Conference

Web site http://nautilus.outreach.uiuc.edu/conted/

New Delhi, **India**
8–12 January 2003

2nd International Congress of Plant Physiology On Sustainable Plant Productivity Under Changing Environment

Web site http://www.ispponline.org/

Ithaca, New York, **USA**
12–17 January 2003

Intellectual Property Management, Agri-Biotechnology Transfer and Trade in an International Context

Web site http://www.swiftt.cornell.edu/

Spokane, Washington, **USA**
14–16 January 2003

Spokane Ag Expo 2003

Web site http://www.agshow.org/

Austin, Texas, **USA**
25–28 January 2003

42nd Vegetable and Flower Seed Conference

Web site http://www.amseed.com/meeting.asp

Ventura, California, **USA**
26–31 January 2003

Gordon Research Conference on Temperature Stress in Plants

Web site http://www.grc.org/03sched.htm

Christchurch, **New Zealand**
2–7 February 2003

ICPP (International Congress of Plant Pathology)

Web site http://events.lincoln.ac.nz/icpp2003/

Christchurch, **New Zealand**
2–7 February 2003

8th International Congress of Plant Pathology

Web site http://events.lincoln.ac.nz/icpp2003/

Lafayette, Louisiana, **USA**
27–30 April 2003

American Forage and Grassland Council Annual Meeting

Web site http://www.afgc.org/me03001.html

Beijing, **China**
6–11 July 2003

XVth International Plant Protection Congress
Ms. Wen Liping, Secretariat, 15th IPPC, c/o Institute of Plant Protection, Chinese Academy of Agricultural Sciences Beijing 100094 China

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

Editor Alok Adholeya **Associate Editor** Subrata Deb **Assistant Editor** Nandini Kumar

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