Vol. 15 No. 4 January 2004

## About TERI

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

# The Bioresources and Biotechnology Division

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

# The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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



# Contents

Effect of soil moisture on arbuscular mycorrhizal developr in plants. Part 1. In grasses, cereals, and fodder crops <i>Sujan Singh</i>		News from members ICOM (International Conference on Mycorrhizae) IV: an Indian perspective	15
<b>Research findings</b> Occurrence of vesicular arbuscular mycorrhizal fungi in		Commercial production of AMF through industrial mode and its large-scale application	15
Marsilea minuta L.	11	Centre for Mycorrhizal Culture Collection Interactions between arbuscular mycorrhizal fungi and	
Status of VAM fungi in lime quarries	13	plant-growth promoting rhizobacteria	16
New approaches	14	Recent references	18
		Forthcoming events	20

# Effect of soil moisture on arbuscular mycorrhizal development in plants. Part 1. In grasses, cereals, and fodder crops

#### Sujan Singh\*

TERI, Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi - 110 003, India

Arbuscular mycorrhizal fungi may increase the resistance of plants to drought by a number of mechanisms, such as increased root hydraulic conductivity, stomatal regulation, hyphal water uptake, and osmotic adjustment (RuizLozano and Azcon 1995). AM (arbuscular mycorrhizae) are also beneficial to plants in waterlogged conditions. The effect of waterlogging on AM development is apparently mediated by physiological responses of the host and by application of hormones such as gibberellic acid or gibberellic acid plus benzyladenine. Under waterlogged conditions AM may directly affect plant growth and the process of root nodulation (especially in legumes) through nutrient transportation, through augmented root surface, etc. AM may also improve plant growth and root nodulation under waterlogged conditions through production of phytohormones (Bano 1987). The present write-up highlights the work done regarding the effect of soil moisture on development of AM on grasses, cereal, and fodder crops. The effect of soil moisture on mycorrhizal development in trees was highlighted in an earlier issue of Mycorrhiza News (Singh 2001).

### Effect of soil moisture on vesiculararbuscular mycorrhiza development in grasses

In studies conducted at the Department of Biological Sciences, Illinois State University, Normal, Illinois, USA, prairie cordgrass (*Spartina pectinata*), a mycorrhizal and typically wet habitat plant, was subjected to five experimentally induced soil moisture conditions. These conditions ranged from continuous saturation to allowing soil

In further studies conducted at the University, Little Bluestem (*Schizachyrium scoparium*) plants were grown in sterilized soil (non-mycorrhizal) or in unsterilized soil (mycorrhizal). The plants were then subjected to varied soil-moisture conditions such as saturation for 12 hours followed by drainage for 48 hours and then allowing the soil to dry to permanent wilting point and returning it to field capacity after 72 hours. With a decrease in soilwater availability, there was a decrease in the percentage of mycorrhizal colonization. Mycorrhizal plants had significantly higher tissue concentrations of phosphorus, calcium, zinc, iron, copper, aluminium, and sodium; and lower concentrations of potassium and manganese than non-mycorrhizal plants. However, non-mycorrhizal plants produced significantly greater root and shoot biomass (P = 0.05), had lower root-shoot ratios, and produced more flowering culms than mycorrhizal plants (Cerligione, Liberta, and Anderson 1988; Liberta and Cerligione 1987).

moisture tensions to reach 15 bars and then saturating the soil 48 hours later. Colonization by VAM fungi occurred under all experimental conditions but was significantly greater under 24-hour saturation followed by 48 hours of drainage, than in any other moisture condition. There was a significant negative correlation between VAM fungal colonization and the weight of the rhizome-tiller units used to propagate experimental plants. There was no significant correlation between measured plantgrowth response and VAM colonization. Plant growth was also not significantly affected by the treatment conditions (Anderson, Ebbers, and Liberta 1986).

<sup>\*</sup> Compiled from TERI database - RIZA

Studies conducted at the Department of Range Science and Ecology Centre, Utah State University, Logan, Utah, USA, on Agropyron smithii and A. dasystachyum, during a wet year, showed that in A. smithii mycorrhizal inoculation decreased stomatal resistance and increased leafwater potential compared to the non-inoculated plants, but only during the driest portion of the growing season. The presence of annuals caused increased stomatal resistance in non-inoculated plants but had no effect on VAM-inoculated plants. This indicates that mycorrhizae may alleviate the detrimental effects of competition on stomatal resistance of A. smithii. In A. dasystachyum, neither VAM inoculation nor annuals had a significant effect on water relations. VAM inoculation also did not increase nitrogen and phosphorus concentrations or the percentage coverage of either grass, but both had delayed phenology when inoculated with the mycorrhiza (Allen and Allen 1986).

Studies conducted at the Department of Botany, University of Wyoming, Wyoming, USA, on *A. smithii* inoculated with two geographically different isolates of *Glomus macrocarpum* var. *macrocarpum* and *G. microcarpum* showed that when compared to non-mycorrhizal plants, leaf resistance to water vapour loss was about 11% lower in all mycorrhizal plants at high soil- and plant-water potentials (-0.2 MPa), and up to 47% lower at soiland plant-water potentials near -6 MPa. Plants infected with *G. microcarpum* from the dry Wyoming site had statistically different responses to drying soil than did plants infected with *G. microcarpum* collected from the more mesic site (Stahl and Smith 1984).

Studies conducted at the Department of Plant Pathology, Kansas State University, Kansas, USA, on Sudan grass (Sorghum vulgare) and Big Bluestem (Andropogon gerardii) showed that the growth of mycorrhizal Big Bluestem was significantly greater than non-mycorrhizal Big Bluestem, even under severe drought stress. However, drought-stressed mycorrhizal plants without phosphorus amendments were not larger than droughtstressed, non-inoculated, phosphorus fertilized (15 mg/kg P) plants, suggesting no increase in drought tolerance. The ability of Glomus etunicatum to benefit plant growth under drought stress was apparently plant mediated and possibly related to the dependency of the plant on mycorrhizal fungus. However, VAM inoculation had no effect on the growth of Sudan grass when cyclic drought stress was imposed on these plants, though the Sudan grass plants benefited from mycorrhizal infection under well-watered conditions when the plants become 1.13-times larger than non-inoculated plants. Inoculated Big Bluestem was 6.56-fold larger than non-inoculated control plants under well-watered condition (Hetrick, Kitt, and Wilson 1987).

#### Effect of soil moisture on vesiculararbuscular mycorrhiza development in cereals

#### Zea mays (maize)

Studies conducted at the Department of Microbiology, GB Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India, on water-stressed maize (grown on sterilized and unsterilized soil and inoculated or not inoculated with introduced VAM fungus [Glomus caledonium] or indigenous VAM population) showed that leafwater potential at different levels of osmotic stress was nearly of the same order in both mycorrhizal and non-mycorrhizal plants. Photosynthetic and photorespiratory activities decreased with increasing osmotic stress but the chlorophyll content remained similar. There was no significant increase in photosynthesis under different treatments and soil conditions in unstressed plants, but it increased significantly in G. caledonium and indigenous VAM + G. caledonium-treated plants than in other treatments. Some increase in chlorophyll (a) and (b) contents was observed in G. caledonium, indigenous VAM + G. caledonium, and phosphorusamended unstressed plants. At osmotic potentials of 0 bar, -2 bars, -5 bars, and -10 bars (leaf-water potentials of -3 bars, -5 bars, -8 bars, and -12 bars, respectively), free proline content increased markedly with decrease in leaf-water potential. This was greatest in phosphorus-amended, nonmycorrhizal plants, followed by G. caledonium, and then indigenous VAM + G. caledonium-inoculated plants (Ramakrishnan, Johri, Gupta 1988a, 1988b)

In further studies at the G B Pant University, five-week-old maize plants (non-VAM and VAM plants inoculated with G. caledonium) were exposed to osmotic potentials of 0 bar, -2 bars, -5 bars, and -10 bars. After eight hours of exposure, leaf-water potentials were about -3 bars, -5 bars, -8 bars, and -12 bars in both VAM and non-VAM plants. Net photosynthesis did not significantly increase at 0 bar, -2 bars, and -5 bars osmotic potentials, but at -10 bars osmotic potential, there was a significant increase in the assimilation of carbon dioxide by the VAM plants (10%-36%). On lowering the water potentials, the rate of decrease in the assimilation of carbon dioxide in VAM plants was very little as compared to the rate of decrease in non-VAM plants. However, among the VAM and non-VAM plants, the differences in the total chlorophyll content were not significant at all the low osmotic potentials (Ramakrishnan, Johri, and Gupta 1990).

Studies conducted at the Department of Biological Science, the University of Dunde, UK, on maize, inoculated with a range of VAM fungi and grown under water-stressed and unstressed conditions, showed that water stress reduced plant growth and this effect was not altered by mycorrhizal infection. However, VAM infection levels were not affected by water stress. Spore production from most inocula, both in total spore's number and in terms of spores per gram plant weight, was reduced by water stress. Sporulations of *Glomus clarum*, *G. epigaeum* (*G. versiforme*), and *G. monosporum* were less affected by water stress than the other inocula (Simpson and Daft 1990).

In studies conducted at the Institut fur Pflanzenernahrung, Justus Liebig Universität, Giessen, Germany, maize was grown in pots on sterilized, sandy, loam soil, with or without *Glomus* etunicatum inoculation and with 340-mg or 850-mg phosphorus per pot (6.5-kg soil) at 70% of maximum water capacity for 33 days. After 33 days, half the pots were kept at 30% of maximum water capacity for eight days, after which normal watering was resumed. Harvest of plants after 33 days, 42 days, and 47 days showed that under water stress, VAM plants took up less phosphorus than control plants, especially at the high phosphorus rate. During the recovery period, the previously stressed VAM plants took up significantly more phosphorus than non-mycorrhizal plants. At the end of the stress period, proline content was lower in VAM plants than in the controls at both, the phosphorus levels under water stress and at the high phosphorus levels without water stress (Muller and Hofner 1991).

In field studies conducted at the Soil and Water Science Department, University of Florida, Gainesville, USA, maize was grown on fumigated Millihopper fine sand (using methyl bromide), which was inoculated or not inoculated with Glomus etunicatum (average rate of 1500 propagules per metre of row placed 10-cm deep in a furrow). The plants were either sufficiently irrigated, moderately water stressed (irrigated after 2-3 days of leaf wilting), or severely water stressed (generally rainfed only). Mycorrhizal colonization was 0%-6%after 6-7 weeks of sowing and 2%-30% after 12-13 weeks of sowing in non-inoculated plants. In inoculated plants, mycorrhizal colonization was 10%-61% after 6-7 weeks and 21%-56% after 12-13 weeks of sowing. Water stress had little effect on root colonization. When total water input increased from 34 cm to 52 cm, biomass increased from 11 000 kg per ha (hectare) to 19 000 kg per ha, while grain yield increased from 4500 kg per ha to 10 200 kg per ha. Inoculated plants had greater biomass (ca. 1000 kg/ha) and grain yield (ca. 800 kg/ha) than uninoculated plants across all water treatments. Due to smaller size of water-stressed plants but, consistent growth response to inoculation across water treatments, the proportional response of corn to inoculation with G. etunicatum increased with increasing water stress (Silvia, Hammond, Bennett et al. 1990; Sylvia, Hammond, Bennett et al. 1993).

In studies conducted at the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria, maize plants were grown in a sterilized, low-phosphorus soil for 12 weeks in a greenhouse and were either inoculated with VAM or given 100-mg or 200-mg soluble phosphorus per kg. Drought stress was imposed after four weeks at weekly intervals. Under stressed conditions, leaf area, shoot dry weight, xylem pressure, soil-water potentials, and total phosphorus uptake were similar for VAM and two phosphorus treatments, but VAMinfected plants had greater root lengths. It was concluded that phosphorus fertilizer application, in addition to mycorrhizal inoculation, may improve drought tolerance of maize plants (Osonubi 1994).

In studies conducted at the Department of Biology, University of Ottawa, Canada, tropical maize from freshly regenerated seeds of selection cycles O (CO) (cv. drought-sensitive) and 8 (C8) (cv. drought-resistant) were grown in a greenhouse. It was inoculated or not inoculated with Glomus *intraradices*, then subjected to drought stress for three weeks following tusselling (75–95 days after sowing), and then was rewatered for the subsequent five weeks until harvest. Mycorrhizal plants of both cultivars had higher leaf-water potential and transpiration rate and lower stomatal resistance throughout the experiment. Green-leaf area was 27.5% higher in mycorrizal than non-mycorrhizal CO plants under drought conditions. Assessment of fully expanded seventh or eighth leaf showed that chlorophyll content was not altered either by water stress or by the presence of mycorrhiza. Mycorrhizal plants had higher total and reducing sugars at the end of the weeks of drought cycle, and had increased protein contents in CO cultivars than non-mycorrhizal plants. Total amino acids increased by 40.6% and 43.7% in non-mycorrhizal plants of CO and C8, respectively. With the presence of AM fungus, amino acid levels increased by 10.7% and 19.2% of leaf dry mass in CO and C8, respectively. Under drought conditions, VAM inoculation enabled the plants to retain considerable amounts of sugars and proteins, especially in CO cultivars. Under drought conditions, mycorrhizal plants had significantly higher uptake of N, P, K, Mg, Mn, and Zn into grain than non-mycorrhizal plants. VAM inoculation also produced greater shoot masses in CO and C8 regardless of drought stress. In CO, drought stress reduced the shoot mass and grain yield by 23% and 55%, respectively, in non-mycorrhizal plants, while reductions were only 12% and 31%, respectively, in mycorrhizal plants. The emergence of tussles and silks was earlier in mycorrhizal than in nonmycorrhizal plants under drought conditions. Mycorrhizal response was more pronounced under both well-watered and drought conditions in CO than in C8 cultivars (Subramanian, Charest, Dwyer et al. 1995; Subramanian and Charest 1995, 1997).

In studies conducted at the Institute of Botany, University of Basel, Basel, Switzerland, maize plants, non-mycorrhizal and mycorrhizal (60% of root length infected with *Glomus mosseae*), were subjected to moderate drought stress by reduction in water supply. The stress induced a conspicuous increase in the trehalose pool in the mycorrhizal roots, accumulation of glucose and fructose in leaves and roots of non-mycorrhizal plants but not in mycorrhizal plants, and complete disappearance of starch from leaves of both mycorrhizal and nonmycorrhizal plants. Acid invertase activity increased during drought in the leaves of both mycorrhizal and non-mycorrhizal plants, while in the roots, it was unaffected in non-mycorrhizal plants but decreased in the mycorrhizal ones. Without drought stress, trehalase activity was considerably higher in the leaves and roots of mycorrhizal plants than in non-mycorrhizal plants, but during drought it increased conspicuously, primarily in the leaves of non-mycorrhizal plants. A drought-induced accumulation of amino acids and imino acids was found in the roots and leaves of both mycorrhizal and non-mycorrhizal plants and the leaves of mycorrhizal plants accumulated more imino acids than those of non-mycorrhizal plants. (Schellenbaum, Muller, Boller et al. 1998)

Studies conducted at the National Agriculture Research Centre, Tsukuba, Ibaraki, Japan, on maize grown on two soils (one under mycorrhizal crops and the other under non-mycorrhizal crops during the preceeding years) and adjusted to soil-water potentials of -10 (wet) KPa, -50 (moist) KPa, and -63 (dry) KPa, seven days after sowing, showed that AM colonization in maize and AM spores in soil were more in the soils with mycorrhizal plants than in soils with non-mycorrhizal plants during the preceeding years. The influence of the previous crop on AM colonization of maize was pronounced in drier soils. AM colonization of maize, however, improved with increasing soil-moisture status even in soils after nonmycorrhizal crops despite the difference in AM spore population. Influence of the previous crop on maize growth declined markedly with an increase in the soilmoisture status (Karasawa, Arihara, and Kasahara 2000).

In studies conducted at the Hokkaido National Agricultural Experiment Station, Sappora, Japan, maize was grown on sterilized Andosol, inoculated with VAM at concentrations of 0 g/kg, 10 g/kg, and 50 g/kg dry soil from 11 days to 75 days after sowing, and water potentials were adjusted to around -10 (wet) KPa, -50 (moist) KPa, or -63 (dry) KPa moisture. The effect of VAM inoculation on maize growth and phosphorus uptake was distinct in dry soil. The effect, however, was less pronounced with increase in soil-moisture status, despite the wide difference in AM spore population and AM colonization (Karasawa, Takebe, and Kasahara 2000).

## Triticum spp. (Wheat)

Studies on wheat conducted at the Division of Botany and Zoology, Australian National University, Canberra, Australia, showed that during good rainfall years, the crop was good and VAM fungi colonized 40%–70% of crop root length. During low rainfall years, the crop was poor and VAM colonization was 5%–16% in the crops most affected by the drought. Wheat plants adjacent to tree linings exhibited particularly poor growth and low VAM colonization due to the trees competing with the crop for water. The study suggests that VAM fungi had no significant role in alleviating the drought stress experienced by the crop (Ryan and Ash 1996).

Studies on wheat, conducted at the Plant Pathology Laboratory, University of Nebraska, Lincoln, USA, showed that at low phosphorus levels, wheat yields were increased by VAM. With prolonged drought, the frequency of wheat plants producing grain was lower for VAM than for non-VAM plants, but grain yields per plant were similar (Yocom, Boosalis, Flessner *et al.* 1987).

In studies conducted at the Department of Horticulture, Pennsylvania State University, USA, wheat (*Triticum aestivum*) was grown with and without VAM fungus, Glomus etunicatum, under environmentally controlled conditions, and with soil phosphorus concentrations adjusted before plantation so that the mycorrhizal and nonmycorrhizal plants produced have similar leaf areas and root-length densities at the same stage of development. Drought-stress treatments were then initiated. Drought did not effect the amount of mycorrhizal infection. Under well-watered conditions, non-mycorrhizal plants had 28% greater shoot dry weight, slightly greater root length densities, and 39% greater phosphorus acquisition than mycorrhizal plants. Under drought conditions, plant size and tissue phosphorus contents of both mycorrhizal and non-mycorrhizal plants were similar. Mycorrhiza did not affect stomatal behaviour during drought stress, but the plants maintained turgor of recently expanded leaves during severe drought. Mycorrhizal infection did not affect the rates at which the root extracted water from soil, whether soil moisture conditions were at their wettest state, at container capacity, or at the driest extreme when soil potentials ranged from -1.5MPa to -2.0 MPa and the plants were completely wilted. Roots extracted water at the fastest rate from the upper soil layers when soil water contents were higher and later extracted water primarily from deeper depths as water in the upper soil layers was depleted. After the drought stress was induced, mycorrhizal infection did not affect changes in leaf water, osmotic pressure potentials, or osmotic pressure of leaf tissues rehydrated to full turgor. Also, mycorrhizal infection did not directly affect drought recovery when plants were rewatered at a range of soil water potentials from -1 MPa to -4 MPa (Bryla and Duniway 1997a, 1997b, 1997c).

Studies conducted at the Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordon, on two wheat (*Triticum*  *durum*) genotypes grown in a low phosphorus (4) mg/kg) silty clay soil (pH 8.1), showed that shoot and root dry matter, total root length, and AM root colonization were higher under well-watered conditions than under dry soil conditions. The droughtresistant genotype CR057 had higher mycorrhizal colonization than drought susceptible genotype CR006. Enhancement in the total dry matter due to mycorrhizal colonization was 42% and 39% under well-watered, and 35% and 45% under waterstressed conditions for CR057 and CR006 genotypes, respectively. For both genotypes, contents of P, Zn, Cu, Mn, and Fe were higher in mycorrhizal than in non-mycorrhizal plants. Enhancement of the uptake of these elements due to AM colonization was more in well-watered than in dry soil and was more pronounced in CR006 than in CR057, particularly under water-stressed conditions. The AM plants had higher water-use efficiency than non-AM plants when grown under water stress, and genotype CR057 had generally higher water-use efficiency values than CR006 (AlKaraki and AlRaddad 1997; AlKaraki and Clark 1998).

Further studies conducted at the above University on wheat (*Triticum durum*) plant showed that *Glomus mosseae* had greater root colonization than *G. monosporum* under both well-watered and waterstressed conditions. Also, *G. mosseae* plants had higher shoot but not root dry matter than *G. monosporum* plants grown under either soil moisture conditions. The *G. mosseae* plants grown under non-water stress generally had higher nutrient contents than *G. monosporum* plants, but nutrient contents were similar for both *G. mosseae* and *G. monosporum* plants grown under water-stress conditions (AlKaraki, AlRaddad, and Clark 1998).

Further studies conducted at the University on one drought-resistant and the other drought-tolerant variety of wheat grown on low phosphorus silty clay soil under water-stressed and well-watered conditions showed that values of benefit or cost for AMF root associations were higher when the plants were water-stressed and decreased under well-watered conditions. Genotypic differences in calculated costs and benefits were pronounced. Benefit or cost analysis may be helpful in evaluating hostplant genotypes in order to optimize efficiencies of AM symbiosis under different environmental conditions (AlKaraki 1998).

## Sorghum bicolor

In a greenhouse trial at the CIAT, APDO, AEREO, Cali, Colombia, USA, *Sorghum bicolor* plants, mycorrhizal and non-mycorrhizal or with *Glomus macrocarpum*, were grown at three water regimes on two soil types (A and B). From the 29th day onwards, total root length of mycorrhizal sorghum was greater than that of non-mycorrhizal sorghum in moderate and high water-stress conditions. A lower percentage of coarse roots and smaller root length per leaf area was found with mycorrhizal plants at all water regimes in soil A, and only in well-watered conditions in soil B. In general, all root and water relation parameters were less affected by water stress when plants were mycorrhizal (Sieverding 1986).

In a greenhouse pot trial at the Faculty of Agriculture, Somali National University, Mogadishu, Somalia, sorghum (cv. NK-367) seedlings, inoculated with *Glomus intraradices* when seven-days old and irrigated to field capacity for seven days were subjected to three moisture regimes, that is, dry (irrigating when soil-water potential was -0.56 MPa to -1.4 MPa), moderately dry (-0.15 MPa to -0.48 MPa), and wet (-0.03 MPa to -0.06 MPa). Plant dry weight was greater in inoculated plants under all irrigation treatments. Photosynthetic rate and stomatal conductance were significantly greater in VAM-colonized than in noninoculated plants under dry and moderately dry conditions (Ibrahim, Campbell, Rupp *et al.* 1990).

Studies conducted at the Department of Biological Sciences, Dundee University, UK, on sorghum inoculated with a range of mycorrhizal fungi and grown under water-stressed and unstressed conditions showed that water stress reduced plant growth of both mycorrhizal and non-mycorrhizal plants. VAM infection levels were not affected by water stress. Spore production by most inocula was reduced by water stress, both in total spore numbers and in terms of spores per gram of plant weight. Sporulations of *Glomus clarum*, *G. epigaeum*, and *G. monosporum* were affected less by stress than were other inocula. Spore production, in general, was greater in sorghum than in maize (Simpson and Daft 1990).

In greenhouse studies conducted at the Institute of Agriculture, University of Tennessee, Knoxville, USA, seedlings of Sorghum bicolor were grown with root system split into four pots. Treatments were applied in a 2 (colonized or not with Glomus intraradices)  $\times$  2 (roots severed or dried)  $\times$ 4 (roots in 0 pot, 1 pot, 2 pots, or 3 pots dried or severed) experimental design. Plants with roots in three pots, dried or severed, showed reduced leaf elongation, stomatal conductance, and leaf-water potential compared with fully watered control plants, and thus probably were hydraulically affected by root treatment. Drying or severing roots in one pot did not affect leaf elongation or stomatal conductance in both mycorrhizal or nonmycorrhizal plants. In non-mycorrhizal plants having two pots, dried, final leaf area and total leaf length were reduced by 18% and 10%, respectively, relative to controls. Stomatal conductance remained unchanged suggesting that decrease in leaf area was not hydraulically induced. Non-mycorrhizal plants having roots severed in two pots continued to have leaf growth similar to control suggesting that growth reduction in the plants

did not result from reduction in root-water gathering capacity. Mycorrhizal symbiosis appeared to eliminate inhibition of root growth that was not hydraulically induced, and leaf area and total leaf length were not reduced in half-dried mycorrhizal plants relative to controls. However, final leaf area of mycorrhizal plants having one or two pots dried was negatively correlated with the product of drying root mass, and the time for which the roots were exposed to mild drought, suggesting that the mycorrhizal plants were also susceptible to growth inhibition that was not hydraulically induced (Ebel, Stodola, Duan *et al.* 1994).

In studies conducted at the Department of Botany and Microbiology, University of Ibadan, Nigeria, sorghum plants were grown in a sterilized, low-phosphorus soil in a greenhouse for 12 weeks and were either inoculated with VAM fungi or given 100-mg or 200-mg soluble P per kg. Drought stress was imposed after four weeks at weekly intervals. Under stress conditions, leaf area, shoot dry weight, xylem pressure, and soil water potentials were similar for VAM and two non-VAM, P-fertilized treatments, but VAM plants had greater root lengths. Total P uptake was higher in VAM than in non-VAM plants. Under drought-stressed conditions, growth parameters and soil-water potential were reduced by VAM inoculation, and greater water extraction occurred in drought-stressed VAM plants. Total P uptake and P uptake per unit root length were significantly enhanced in nonmycorrhizal, P-fertilized treatments compared with the mycorrizal treatments (Osonubi 1994).

Studies conducted at the Plant Pathology Laboratory, University of Nebraska, Lincoln, USA, on sorghum grown in the greenhouse with or without *Glomus deserticola*, and under normal and reduced watering schedules initiated one month before planting, showed that the level of VAM colonization was reduced by water stress. Also, VAM did not increase yield at any P level and did not interact with water treatments to increase yield of VAM plants (Yocom, Boasalis, Flessner *et al.* 1987).

#### Oryza sativa (Rice)

In studies conducted at the Laboratory of Plant Nutrition, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Japan, wetland rice plants grown in two soils (original paddy soil and paddy soil + Andosol 1:4) inoculated or not with VAM (mainly *Glomus* sp.), were subjected to four water regimes. These were (1) flooded up to maturity (F), (2) non-flooded (kept at 60% moisture of maximum water-holding capacity up to maturity (NF), (3) flooded for three weeks after sowing and then non-flooded up to maturity (F-NF), and (4) non-flooded for three weeks after sowing and then flooded up to maturity (NF-F). Grain formation was improved under F and NF-F than under non-flooded (NF and F-NF) conditions. Grain formation was retarded in the latter resulting in low harvesting index, especially in inoculated plants. Mycorrhizal treatments resulted in a decrease in the amount of shoot dry matter under flooded conditions. Though mycorrhizal colonization was highest under nonflooded conditions, 2%-21% colonization was observed even under flooded conditions at 60 days after transplanting and 3% remained in the NF-F treatment, whereas no colonization occurred under continuously flooded conditions (F) up to the maturation stage. VAM colonization significantly increased N concentration in unhulled grain, significantly decreased shoot N concentration, and did not affect P concentration at the maturation stage (Solaiman and Hirata 1995).

#### **Fodder crops**

#### Medicago sativa (alfalfa)

In studies conducted at the Navarra University, Pamplona, Spain, *Medicago sativa* plants, grown in P-deficient soil and inoculated or not with *Glomus mosseae* and/or *Rhizobium meliloti*, were subjected to water stress down to -0.7 MPa of soil-water potential after 40 days. *G. mosseae* increased nitrogenase activity and N fixation where leaf-water potential was -0.5MPa, and also caused changes in stomatal conductance, photosynthesis, phosphorus uptake, and rootshoot dry weight ratio. Tripartite symbiosis appeared to adapt to water stress conditions (Pena 1985).

Further studies conducted at the University (Deparmtento de Fisilogia Vegetal) on Medicago sativa plants, both mycorrhizal nodulated and P-compensated nodulated, under both well-watered and drought-acclimatized conditions, showed that leaf-area ratio significantly decreased in P-fertilized plants in response to drought treatment, whereas mycorrhizal plants maintained fairly constant values. As stress progressed, VAM plants developed lower root-shoot and higher leaf area ratios than non-VAM plants. Total dry weight decreased for both treatments in response to drought, but the reduction was more pronounced in P-fertilized plants. Under watered conditions, CO<sub>2</sub> exchange rate and photosynthetic P-use efficiency were approximately 30% and 55% higher, respectively, in VAM than in non-VAM plants, while specific nodule activity was similar in both groups of plants. Throughout drought, CO<sub>2</sub> exchange rate and photosynthetic P-use efficiency maintained significantly higher value in mycorrhizal plants. Internal CO<sub>2</sub> concentration was always higher in P-compensated than in VAM plants (Sanchez-Diaz, Pardo, Antolin et al. 1990).

In further studies conducted at the University, Medicago sativa plants were inoculated or not with Glomus fasiculatum and or Rhizobium meliloti (with non-VAM and non-rhizobium plants provided with equivalent P and N, respectively, to obtain same growth), and were subjected to two cycles of drought and recovery, sixty days after planting. Nodulated plants with only Rhizobium (R) and Rhizobium + VAM (MR) had the lowest ABA concentrations in roots under well-watered conditions. Water stress increased ABA concentrations in leaves of control (N), R, and MR plants while ABA concentrations in only VAM (M) plants did not change. The highest production of ABA under water deficit was in the roots of non-mycorrhizal plants. Under water stress, the ratio of ABA to cytokinin concentrations strongly increased in leaves and roots of N plants, decreased in the roots of M plants, and remained unchanged in leaves and roots of MR plants. Photosynthesis, leaf conductance, and the transpiration rate decreased in all treatments with strongest decrease in N plants when stress was imposed (Goicoechea, Antolin, and Sanchez Diaz 1997).

### Trifolium spp (Clover)

Studies conducted at the Department of Biology, University of York, UK, on *Trifolium pratense*, grown in pots in a factorial design with four rates of P and with or without mycorrhizal inoculum (*Glomus spp.*), showed that under well-watered conditions, only uninfected plants on very low P soil showed reduced stomatal conductance and the lowest leaf-P concentration. During drought, only plants with very high leaf-P concentration maintained high conductance. There was no evidence of increased water uptake by mycorrhizal plants. This suggested that mycorrhizal effects on water relations are secondary consequences of changes in P nutrition (Fitter 1988).

In studies conducted at the Soil Science and Plant Nutrition, the University of Western Australia, Australia, clover plants were grown on steam-sterilized, low-phosphorus soil, and inoculated with Acaulospora laevis, a Glomus sp., and a fine endophyte in a precycle of four weeks of clover growth to establish a mycelial network or the pots without clover growth were left dry. After the precycle, pots dried to less than 1% water content were either left dry or were treated with a false break in which the soil was watered for three days in a row to field capacity and then allowed to dry to less than 1% soil-water content. All the pots were then planted with clover and maintained at field capacity until 14-28 days after planting when mycorrhizal colonization was assessed. Mycorrhizal colonization by A. laevis was increased by a false break only in pots that were not treated with a precycle. The proportion of root length colonized by A. laevis hyphae, 28 days after planting in a growing season, increased from 16% to 69% in pots treated with a false break. Mycorrhizal colonization by *Glomus sp.* was decreased by both a false break and a precycle, while colonization by fine endophyte was not decreased by a false break but by the precycle (Braunberger, Abbott, and Robson 1993).

In further studies at the University on subterranean clover (Trifolium subterraneum), undisturbed cores were collected in summer from two annual clover-based pastures in Western Australia. The cores were treated with one or two false breaks or were left dry. For each false break, 3 cm of water was applied to the surface of the cores over a threeday period and the cores were left to dry in glasshouse conditions. The second false break and the growing season were initiated after the complete death of plants that had emerged in the previous cycle. Then the growing season was simulated by watering all cores to field capacity and planting to a uniform stand of subterranean clover. Mycorrhizal colonization was decreased by false breaks with little difference between one or two false breaks. In one type of soil, the percentage of root length containing arbuscules at 14 days after planting in the growing season was decreased from 52% to 33% by false breaks, and at 42 days after planting, from 61% to 46%. In the other soil, the arbuscules at 28 days after planting in the growing season decreased from 72% to 55% by false breaks. Assessment of morphology of mycorrhiza revealed that colonization by fine endophytes was decreased but colonization by Acaulospora spp., Glomus spp., Scutellospora *spp.*, *Gigaspora spp.* was not decreased by false breaks (Braunberger, Abbott, and Robson 1994).

## Stylosanthus hamata

Studies conducted at the Postgraduate Department of Botany, Utkal University, Bhubaneswar, Orissa, India, on *Stylosanthus hamata*, grown on degraded forest wasteland soil in the presence of *Jalshakti* (a chemical polymer known for increasing waterholding capacity of soil) showed that there was an indication of retardation of growth, nodulation, and rhizospheric microbial activity with Jalshakti under adequate water conditions (daily watering). Under limiting watering (three-day gap), the effectiveness of AM fungal inoculation, and stimulating growth and nodulation capacity of Stylosanthus was seen to be enhanced significantly in the presence of Jalshakti. There was about 66% of water economy along with induction of drought tolerance capacity due to Jalshakti application and AM inoculation. Also, application of AM fungi in the presence of Rhizobium was seen to be more effective in enhancing water-stress tolerance capability in Stylosanthus, indicated through enhancement of plant growth, biomass nodulation capacity, and rhizospheric microbial activity (Misra, Thatoi, and Mallik 1999).

## References

AlKaraki G N. 1998 Benefit, cost, and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress Mycorrhiza 8(1): 41–45 AlKaraki G N and AlRaddad A. 1997

Effects of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance

Mycorrhiza 7(2): 83–88

AlKaraki G N, AlRaddad A, and Clark R B. 1998 Water stress and mycorrhizal isolate effects on growth and nutrient acquisition of wheat Journal of Plant Nutrition 21(5): 891–902

AlKaraki G N and Clark R B. 1998

Growth, mineral acquisition, and water use by mycorrhizal wheat grown under water stress Journal of Plant Nutrition 21(2): 263–276

Allen E B and Allen M F. 1986

Water relations of xeric grasses in the field: interactions of mycorrhizas and competition New Phytologist 104: 559–571

Anderson R C, Ebbers B C, and Liberta A E. 1986 Soil moisture influences colonization of prairie cordgrass (Spartina pectinata lind.) by vesicular-arbuscular mycorrhizal fungi New Phytologist 102: 523–527

#### Bano A. 1987

Role of vesicular arbuscular mycorrhiza in hydrophytic condition in relation to phytohormone, pp. 431–443

In Mycorrhiza Roundtable: proceedings of a workshop

[JNU-IDRC, Canada sponsored National Workshop on Mycorrhizae, New Delhi, India, 13-15 March 1987]

Braun-berger P G, Abbott L K, and Robson A D. 1993 The effect of false breaks on the formation of mycorrhizas by *Acaulospora laevis*, a *Glomus* sp., and five endophytes, p. 6

In Ninth North American Conference on Mycorrhizae, 8–12 August 1993

Braunberger P G, Abbott L K, and Robson A D. 1994
The effect of rain in the dry-season on the formation of vesicular-arbuscular mycorrhizas in the growing season of annual clover-based pastures
New Phytologist 127(1): 107–114

Bryla D R and Duniway J M. 1997a

Growth, phosphorus uptake, and water relations of safflower and wheat infected with an arbuscular mycorrhizal fungus

*New Phytologist* **136**(4): 581–590

Bryla D R and Duniway J M. 1997b
Water uptake by safflower and wheat roots infected with arbuscular mycorrhizal fungi
New Phytologist 136(4): 591-601

Bryla D R and Duniway J M. 1997c

Effects of mycorrhizal infection on drought tolerance and recovery in safflower and wheat *Plant and Soil* **19**7(1): 95–103 Cerligione L J, Liberta A E, and Anderson R C. 1988 Effects of soil moisture and soil sterilization on vesicular-arbuscular mycorrhizal colonization and growth of Little Bluestem (Schizachyrium scoparium)

Canadian Journal of Botany 66(4): 757-761

Ebel R C, Stodola A J W, Duan X, Auge R M. 1994 Non-hydraulic root-to-shoot signalling in mycorrhizal and non-mycorrhizal sorghum exposed to partial soil drying or root severing New Phytologist 127(3): 495–505

Fitter A H. 1988

Water relations of red clover *Trifolium pratense* L as affected by VA mycorrhizal infection and phosphorus supply before and during drought *fournal of Experimental Botany* **39**(202): 595–603

Goicoechea N, Antolin M C, and Sanchez Diaz M. 1997 Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought

Physiologia Plantarum 100(4): 989-997

Hetrick B A D, Kitt D G, and Wilson G T. 1987 Effects of drought stress on growth response in Corn Sudan grass and Big Bluestem to *Glomus-etunicatum* New Phytologist 105(3): 403–410

Ibrahim M A, Campbell W F, Rupp L A, Allen E B. 1990

Effects of mycorrhizae on sorghum growth, photosynthesis, and stomatal conductance under drought conditions

Arid Soil Research and Rehabilitation 4(2): 99-107

Karasawa T, Arihara J, and Kasahara Y. 2000
Effects of previous crops on arbuscular mycorrhizal formation and growth of maize under various soil moisture conditions
Soil Science and Plant Nutrition 46(1): 53–60

Karasawa T, Takebe M, and Kasahara Y. 2000
Arbuscular mycorrhizal (AM) effects on maize growth and AM colonization of roots under various soil moisture conditions
Soil Science and Plant Nutrition 46(1): 61–67

Liberta A E and Cerligione L J. 1987

Effects of soil moisture on VAM colonization and growth of Little Bluestem, p. 296

[In Proceedings of the Seventh North American Conference on Mycorrhiza, edited by D M Sylvia, L L Hung, and J H Graham, 3–8 May 1987, University of Florida, Florida]

Florida: University of Florida, 364 pp.

Misra A K, Thatoi H W, and Mallik D. 1999 Induction of drought tolerance in Stylosanthes hamata (L.) taub with AM fungi and Jalsakti grown in forest degraded wastes of Chandaka in Orissa [In Fourth National Conference on Mycorrhiza. Section –
 2: Association and distribution of mycorrhizal fungi under varying edapho-climatic conditions, 5–7 March
 1999, Barkatullha University, Bhopal, India]
 Bhopal: Barkatullha University

Muller I and Hofner W. 1991

Influence of VA-mycorrhiza on P uptake and recovery potential of maize (Zea mays L.) under water-stress conditions

Zeitschrift fur Pflanzenernahrung und Bodenkunde 154(5): 321–323

Osonubi O. 1994

Comparative effects of vesicular-arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and phosphorus uptake of maize (Zea mays L.) and sorghum (Sorghum bicolor L.) plants under

Biology and Fertility of Soil 18(1): 55-59

#### Pena C J I. 1985

Physiology of legume-rhizobium-VA mycorrhiza symbiosis under water stress

Dissertation Abstracts International (European abstracts) 46(2): 361–362

Ramakrishnan B, Johri B N, and Gupta R K. 1988a Effect of vesicular arbuscular mycorrhizal fungus on photosynthesis and photorespiration in water-stressed maize

Photosynthetica 22(3): 443–447

Ramakrishnan B, Johri B N, and Gupta R K. 1988b

Influence of VAM fungus *Glomus caledonius* on free proline accumulation in water-stressed maize

Current Science 57(19): 1082-1084

- Ramakrishnan B, Johri B N, and Gupta R K. 1990 The response of mycorrhizal maize plants to variations in water potentials
- In *Current Trends in Mycorrhiza Research*, edited by B L Jalali and H Chand, pp. 61–62

Hisar: Haryana Agricultural University. 210 pp.

[Proceedings of the National Conference on Mycorrhiza, Hisar, India, 14–16 February 1990]

RuizLozano J M and Azcon R. 1995

Hyphal contribution to water uptake in mycorrhizal plants as affected by the fungal species and water status Physiologia Plantarum 95(3): 472–478

1 hystologia 1 tantarian 75(5): 112 1

Ryan M H and Ash J E. 1996

Colonisation of wheat in southern New South Wales by vesicular-arbuscular mycorrhizal fungi is significantly reduced by drought

Australian Journal of Experimental Agriculture 36(5): 563–569

Sanchez-Diaz M, Pardo M, Antolin M, Pena J, Aguirreolea J. 1990

Effect of water stress on photosynthetic activity in the Medicago-Rhizobium-Glomus symbiosis Plant Science (Limerick) 71(2): 215–221 Schellenbaum L, Muller J, Boller T, Wiemken A, Schuepp H. 1998

Effects of drought on non-mycorrhizal and mycorrhizal maize: changes in the pools of non-structural carbohydrates, in the activities of invertase and trehalase, and in the pools of amino acids and imino acids

New Phytologist 138(1): 59-66

Sieverding E. 1986

Influence of soil water regimes on vesiculararbuscular mycorrhiza IV. Effect on root growth and water relations of sorghum-bicolor Journal of Agronomy and Crop Science 157(1): 36-42

Simpson D and Daft M J. 1990

Interactions between water-stress and different mycorrhizal inocula on plant growth and mycorrhizal development in maize and sorghum *Plant and Soil* **121**(2): 179–186

#### Singh S. 2001

Role of mycorrhiza in tree plantings in the field, Part II: field inoculation, fungal succession, and effect of climatic and edaphic factors Mycorrhiza News 12(4): 2–12

Solaiman M Z and Hirata H. 1995

Effects of indigenous arbuscular mycorrhizal fungi in paddy fields on rice growth and N P K nutrition under different water regimes Soil Science and Plant Nutrition 41(3): 505–514

Stahl P D and Smith W K. 1984

Effects of different geographic isolates of glomus on the water relations of *Agropyron* Smith II *Mycologia* 76(2): 261–267

Subramanian K S and Charest C. 1995
Influence of arbuscular mycorrhizae on the metabolism of maize under drought stress
Mycorrhiza 5(4): 273–278

Subramanian K S and Charest C. 1997

Nutritional, growth and reproductive responses of maize (Zea mays L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling

Mycorrhiza 7(1): 25–32

Subramanian K S, Charest C, Dwyer L M, Hamilton R I. 1995

Arbuscular mycorrhizas and water relations in maize under drought stress at tasselling The New Phytologist 129: 643–650

*The New Thylologist* **127**. 045–050

Sylvia D M, Hammond L C, Bennett J M, Haas J H. 1990

Field response of corn to water management and inoculation with *Glomus etunicatum* 

[Proceedings of the Eighth North American conference on Mycorrhiza Innovation and Hierarchical Integration, 5–8 September 1990, Jackson, Wyoming]

Wyoming: University of Wyoming, p. 274

Sylvia D M, Hammond L C, Bennett J M, Haas J H, Linda S B. 1993

Field response of maize to a VAM fungus and water management

Agronomy Journal 85(2): 193-198

- Yocom D H, Boosalis M G, Flessner T R, Cunningham E A. 1987
- The effects of prolonged drought on the yield of mycorrhizal and nonmycorrhizal grain crops,

[In Proceedings of the Seventh North American Conference on Mycorrhiza, p. 296, edited by D M Sylvia, L L Hung, and J H Graham, 3–8 May 1987, Gainesville, Florida Florida: University of Florida, 364 pp.

# **Research findings**

## Occurrence of vesicular arbuscular mycorrhizal fungi in Marsilea minuta L.

#### P Rama Bhat and K M Kaveriappa\*

Department of Applied Botany, Mangalore University, Mangalagangothri – 574 199, Karnataka, India \*E-mail: profkmk@yahoo.com

VAM (vesicular-arbuscular mycorrhiza) has been reported to be associated with a wide range of angiosperms and lower plants (Newmann and Reddell 1987; Mohan Kumar and Mahadevan 1988; Ragupathy and Mahadevan 1993). According to Boullard (1979), fungal endophytes pass from an obligate state in Psilotaceae and Lycopodiaceae to a constant one in eusporangiate ferns, a facultative one in some Leptosporangiate ferns (Polypodiaceae, Pteridaceae, etc.), and in others (those belonging to aquatic families such as Azollaceae, Marsileaeae, etc.) they are completely absent.

VAM infection has been reported in three out of 12 species of aquatic plants (Bagyaraj, Manjunath, and Patil 1979) investigated, including *Salvinia cucullata* Roxb. Newman and Reddell (1987) compiled the information on mycorrhizal status of plants growing in the field and found 97 out of 169 species of pteridophytes forming VAM.

In a survey of pteridophytes in the Coimbatore area, 60–71 pteridophytes belonging to 30 families were found to have VAM association, but in the aquatic and marshy ferns it was absent (Muthukumar and Udaiyan 2000). Out of 14 species of Marsilea reported from India (Baker 1984; Dixit and Vohra 1984), VAM association has been recorded on M. quadrifoliata L. (Firdaus-e-Bareen 1990). The present report is on the association of VAM fungi with the roots and rhizomes of M. minuta, an aquatic fern growing in the marshy habitat of this region in the rainy season, and perennating in the form of rhizomes during summer. The plant is used as a vegetable; in the preparation of medicines for the treatment of cough, spastic conditions of leg muscles, etc.; and as a sedative (Dixit and Vohra 1984).

## Materials and methods

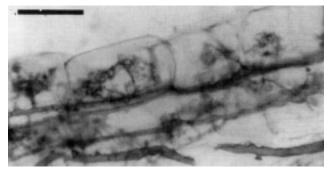
Fine feeder roots and rhizomes of *M. minuta* were collected along with the rhizosphere soil from

Uppala, Kasaragod district, Kerala, during 1997/98. VAM infection was estimated by the techniques of Koske and Gemma (1989). For this, the roots and rhizomes were washed in water to remove adhering soil particles and stored in formalin-aeetoalcohol. Fifty pieces each of rhizomes and roots, each piece 10-mm long, were taken and rinsed two to three times in fresh water. Then they were boiled in 10% KOH at 90 °C for 30–45 minutes, again rinsed in water, acidified in 1% HCL, and stained in acidic glycerol containing 0.05% trypan blue. Stained specimens were examined under a stereo microscope and the intensity of mycorrhizal colonization, including the presence of hyphae, vesicles, and arbuscules, was recorded.

Mycorrhizal spores from rhizosphere soil were isolated by wet sieving and decanting methods (Gerdemann and Nicholson 1963). For this, three samples, each of 100 g of rhizosphere soil, were sieved through a series of wire meshes, having a mesh size 700–45  $\mu$ m. The residues from sieves were transferred to petri dishes containing distilled water. The number of spores present in the residue was counted using a stereo microscope. The rhizosphere soil yielded an average of 290 VAM spores and sporocarp per 100 g of soil. Samples of spores were mounted on slides containing polyvinyl alcohol and sealed. The spores were identified using the appropriate manuals.

#### Results

The rhizomes and roots showed the presence of hyphae and vesicles (Figure 1). Arbuscles were not observed. The mycorrhizal colonization was 60%-70% in both rhizomes and roots. VAM population of 12 species belonged to four genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, and *Sclerocystis*. The maximum number of species belonged to *Acaulospora* and *Glomus*. The species recorded were as follows: *Acaulospora bireticulata* (Rothwell & Trappee), A. laevis (Gerdemann & Trappee), A. scrobiculata Trappee, A. trappei (Ames & Linderman), Gigaspora decipiens (Hall & Abbott), Glomus aggregtum (Schenck & Smith) emend. Koske, G. albidum (Walker & Rhodes), G. fasciculatum (Thaxter) (Gerdemann & Trappee), G. geosporum (Nicholson & Gerdemann) Walker, G. macrocarpum (Tulasne & Tulasne), G. monosporum (Gerdemann & Trappe), and Sclerocystis indica (Bhattacharjee & Mukerji). The spores of Glomous monosporum, G. fasciculatum, and A. scrobiculata species were dominant.



**Figure 1** A portion of the root of *Marsilea minuta* showing hyphae and vesicles (X20)

## Discussion

Earlier investigators either did not observe endomycorrhiza on aquatic plants or where such observations were made, the frequency of colonization was poor (Read, Kouchiki, and Hodgson 1976; Bagyaraj, Manjunath, and Patil 1979). However, some recent reports have shown that mycorrhizal colonization is common among aquatic plants (Firdaus-e-Bareen 1990; Dharmarajan, Kannan, and Lakshminarasimhan 1993). A low colonization rate of 12%-20% in some pteridophytes of Sakleshpur, Karnataka; and 25%-75% in the Nilgiri and Kodaikanal hills of Tamil Nadu, has been recorded (Raja, Ragupathy, and Mahadevan 1995; Mallesha, Bagyaraj, and Joshi 1996). Among the pteridophytes of Coimbatore, Tamil Nadu, a wide range of colonization (15%–70%) was reported, but colonization was not observed in Marsilea minuta (Muthukumar and Udaiyan 2000). In the present investigation, as many as 12 species of VAM fungi were found in the rhizosphere of M. minuta of which the spores of three species, Glomus monosporum, G. fasciculatum, and A. scrobuculata, were dominant. Spores of nine VAM species, belonging to Acaulospora, Glomus, Sclerocystis, and Scutellospora, were recorded in the pteridophytes of Coimbatore region (Muthukumar and Udaiyan 2000); nine VAM species were isolated from the rhizosphere of ferns of Kodaikanal; and 13 species from ferns of Nilgiri hills belonging to the genera Acaulospora, Gigaspora, Glomus, and

Sclerocystis (Raja, Ragupathy, and Mahadevan 1995). It is believed that the VAM fungi may be playing some role in nutrient absorption, especially in plants growing in nutritionally poor conditions (Firdaus-e-Bareen 1990).

## Acknowledgement

The authors are grateful to Kudremukh Iron Ore Company Ltd, Bangalore, for providing financial assistance to carry out the investigations.

### References

Bagyaraj D J, Manjunath A, and Patil R B. 1979 Occurrence of vesicular arbuscular mycorrhizas in some tropical aquatic plants

Transactions of the British Mycological Society 72: 164-167

#### Baker J G. 1984

A synopsis of the genera and species of the natural orders—*Equisetaceae*, *Lycopodiaceae*,

Selanginellaceae, and Rhizocarpaceae In Handbook of the Fern-allies

Dehra Dun: Bishen Singh Mahendra Pal Singh, pp. 139–141

#### Boullard B. 1979

Considerations sur la symbiose Fongique Chez les Pteridophytes, Syllogens No. 19, pp. 1–58

Ottawa, Canada: National Museum of Natural Sciences

Dharmarajan S, Kannan K, and Lakshminarasimhan C. 1993

Vesicular-arbuscular mycorrhizal status of some aquatic and marshy plants

Acta Botanica Indica 21: 167–171

Dixit R D and Vohra J N. 1984 A dictionary of the pteridophytes of India, p. 28 Howrah: *Botanical Survey of India* 

Firdaus-e-Bareen. 1990

**Vesicular arbuscular mycorrhiza in aquatics,** pp. 1–3 In *Current Trends in Mycorrhizal Research* 

[Proceedings of the National Conference on Mycorrhizae, Haryana Agriculture University, Hissar, 14–16 February 1990, edited by B L Jalali and H Chand.]

Gerdemann J W and Nicholson T H. 1963 Spores of mycorrhizal *Endogone* species isolated by wet-sieving and decanting

Transactions of the British Mycological Society 46: 235-244

Koske R E and Gemma J N. 1989 A modified procedure for staining roots to detect VA mycorrhizas

Mycological Research 92: 486-489

Mallesha B C, Bagyaraj B J, and Joshi S S. 1996 Occurrence of vesicular arbuscular mycorrhizal fungi in pteridophytes

Journal of Soil Biology Ecology 16: 169–170

Mohan Kumar V and Mahadevan A. 1988 Ragupathy S and Mahadevan A. 1993 Studies on vesicular arbuscular mycorrhizal Distribution of vesicular arbuscular mycorrhizae association in plants of Kalakad reserve forests in the plants and rhizosphere soils of the tropical Journal Indian Botanical Society 67: 41-46 plants Mycorrhiza 3: 123-136 Muthukumar T and Udaiyan K. 2000 Vesicular arbuscular mycorrhizae in pteridophytes Raja P, Ragupathy S, and Mahadevan A. 1995 of Western Ghats, south India A mycorrhizal association of pteridophytes of Phytomorph 50: 132-142 Nilgiri and Kodaikanal hills, south India Acta Botanica Indica 23: 181–186 Newmann E I and Reddell P. 1987 The distribution of mycorrhizas among families of Read D J, Kouchiki H K, and Hodgson J. 1976 vascular plants Vesicular arbuscular mycorrhiza in natural The New Phytologist 106: 745-751 vegetation system The New Phytologist 77: 641-653

## Status of VAM fungi in lime quarries

Naveen Malaviya and Jamaluddin\*

Assistant Professor of Botany, Government Science College (Institute of Excellence), Jabalpur

VAM (vesicular-arbuscular mycorrhiza) fungi plays an important role in growth and development of vegetation in the forest and other localities. Excavation of lime in the Katni district of Madhya Pradesh is done regularly. Due to mining, considerable amount of vegetation is disturbed and the ground is exposed to the sun causing soil dessication. The water-holding capacity of soil is also less in limestone sites. Due to such mining activities, the microbial population including VAM fungi was studied in three different sites of the Katni district and compared with the VAM flora occurring in the existing degraded forests near mined areas.

Three different sites in Katni were selected where lime quarries are existing. Given below are the study sites and the status of existing vegetation noticed there in the month of February–March 2002 (Table 1).

Site-I Devari Hatai This site is placed in a diversion just before Katni (at Jabalpur–Katni Road). The lime quarries were abandoned and excavation was almost stopped. The vegetation is rich in grasses, Lantana camara, Cassia fistula, Butea monosperma, and Lagerstroemia parviflora. Few cultivated trees were also observed.

Site-II Roadside route between Katni and Jhukehi The belt was full of lime furnaces crossing the Jhukehi station; there was a small hillock from where lime is still excavated. Grasses were common and under shrubs like Lantana camara were noticed. Amongst the small trees, Cassia fistula and Butea monosperma were common in the roadside. Few trees were also observed on the hillock. Site III Kymore Road From Katni to Kymore, few mines are there. The vegetation was comparatively rich, comprising Butea monosperma, Cassia fistula, Lagerstroemia parviflora, and grasses.

Soil samples from the above three sites were collected in polybags. The samples were also collected from the nearby degraded forest. VAM spores were extracted by Gerdemann and Nicolson (1964) techniques, widely known as wet sieving and decanting techniques. Spores were studied by adopting the techniques used by Chandra and Jamaluddin (1999). After isolation, the VAM consortium was grown in pure lime soil + lime soil and normal soil (in the ratio of 50:50). *Panicum maximum* grass was used as the trap plant. The population of spores and root infection in *P. maximum* were recorded after three months.

It is evident from the Table 1 that VAM flora was less in lime quarries as compared to the adjoining degraded forests. The frequency of VAM spores was also less in lime mines. All the three sites contain a wide variety of VAM flora. *Glomus geosporum* was absent in site III. Comparatively, *Glomus mosseae*, *Gigaspora*, *Scutellospora* were in high frequency in all the sites. The spore population was more in site III as compared to site I and II. Chandra and Jamaluddin (1999) also recorded less VAM fungi in coalmines over dumps compared to natural forest areas.

It was observed that isolated VAM flora grow well in pure soil and also in lime soil amended with normal soil. The population of spores in both types of soil did not show much variations in per cent root infection in *Panicum maximum*, which was 45%–50%. This study indicated that the VAM

\*Head, Department of Forest Pathology, Tropical Research Forest Institute, Jabalpur

 Table 1
 The status of vesicular-arbuscular mycorrhiza in three
 different lime-mined areas and in degraded forests

Vesicular-arbuscular mycorrhiza species	Site I	Site II	Site III	Degraded forests
Glomus mosseae	20	18	26	25
G. fasciculatum	11	10	_	21
G. geosporum	_	_	7	13
Glomus sp.	3	_	5	14
Acaulospora scrobiculata	9	12	11	19
Gigaspora margarita	8	18	21	22
Scutellospora sp.	19	15	18	20

consortium obtained from such harsh sites was suitable as inoculum for afforestation in lime-mined areas. Further studies on suitability of VAM inocula for lime-mined areas is in progress.

#### Acknowledgement

The authors are thankful to the Central Regional Office, University Grants Commission, Bhopal, for providing financial assistance.

#### References

Chandra K K and Jamaluddin. 1999 Distribution of vesicular arbuscular mycorrhizal fungi in coalmines over burden dumps

Indian Phytopathology 52(3): 254–258

Gerdemann J W and Nicolson T H. 1964 Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting

Transactions of the British Mycological Society 46: 235-244

# New approaches

Ectomycorrhiza development alters gene expression in the fungal and plant symbionts. Identification of a large number of genes expressed exclusively or predominantly in the symbiosis will contribute greatly to an understanding of the development of ectomycorrhizal symbiosis. Voiblet C, Duplessis S, Encelot N, and Martin F constructed a cDNA library of four-day-old *Eucalyptus globules–Pisolithus tinctorius* ectomycorrhiza and sequenced 850 DNAs cloned randomly or obtained through SSH (suppression subtractive hybridization) (*Plant Journal* **25**(2):181–191, 2001).

Based on the absence of a database match, 43% of the ectomycorrhiza ESTs are coding for novel genes. At the developmental stage analysed (fungal sheath formation), majority of the identified sequences represented 'housekeeping' proteins, that is, proteins involved in gene protein expression, cell wall proteins, metabolic enzymes, and components of signalling systems. We screened arrayed cDNAs to identify symbiosis-regulated genes by using differential hybridization. Comparisons of signals from free-living partners and symbiotic tissues revealed significant differences in expression levels (differential expression ratio >2.5) for 17% of the genes analysed. No ectomycorrhizaspecific gene was detected. The results successfully demonstrate the use of the cDNA array and SSH systems as general approaches for dissecting symbiosis development, and provide the first global picture of the cellular functions operating in ectomycorrhiza.

# News from members

Dr Alok Adholeya participated in ICOM (International Conference on ycorrhiza) -IV held at Montreal, Canada, during 10–15 August 2003. He delivered a talk on *Commercial production of AMF through industrial mode and its large-scale application* as a special invitee in the conference.

Dr Alok Adholeya also visited various laboratories at Basel and Neuchatel in Switzerland to exchange scientific knowledge.

Abstract of the lecture delivered by Dr Alok Adholeya was part of *In vitro biology of mycorrhizal fungi*, (Symposium 10.) session of ICOM-IV.

# ICOM (International Conference on Mycorrhizae) IV: an Indian perspective

The Fourth International Conference on Mycorrhizae was a prime source of information on mycorrhizal symbiosis. The Conference, held in Montreal, Canada, during 10-15 August 2003, was important as it gathered the scientific community working on a common subject and with varied and vast expertise, to interact, communicate, and exchange their findings and impressions on the issue of complex mycorrhizal symbiosis. The conference theme referred to the basic role of mycorrhizae in the evolution of life, plant species, land ecosystems, and the multiple benefits man can derive from their use. Experts from different disciplines participated, reflecting their experiences ranging from mycorrhizal fundamentals, ecology, soil sciences, forestry, agronomy, taxonomy, molecular biology, physiology, biochemistry, signalling, and related fields. The basic role of mycorrhizae in the evolution of life, plant species, land ecosystems, and the multiple benefits man can derive from their use were the key issues discussed.

The Conference provided an opportunity for networking with colleagues, scientists, agronomists, industry people, and other professionals from every continent. The event was unique in itself with the involvement of two main prestigious societies, the Canadian Society of Agronomy and the Canadian Society of Soil Sciences, which are responsible for organizing annual scientific meetings across Canada. Approximately, 750 abstracts were presented in the form of different symposiums, workshops, and oral and poster sessions.

Several Indian scientists, both residing in India and elsewhere, participated in the events. The major participation from India was from TERI-several abstracts were presented by the group representing TERI along with major oral presentations on the technologies developed and commercialized on mycorrhiza mass production. The presentations described the overall process of technological developments along with specific benchmarks designed for both high quality and quantity production of mycorrhizal propagules. The presentations highlighted the various features of production where several optimizations and formulations have been developed (the product has been validated by an enduser survey and multiple field demonstrations were conducted to evaluate its efficacy). The presentations were highly appreciated globally since such technology presently does not exist elsewhere.

Another participation from the TERI delegates was on the investigation of tolerance of ecto mycorrhiza with different elements when amended in the media. The group from Y S Parmar University of Horticulture & Forestry, Himachal Pradesh, presented an abstract on the occurrence and distribution of arbuscular mycorrhiza fungi in citrus in north-western Himalayan region of India with diverse management systems.

Considering India's agriculture-based economy and large dependence of rural masses on agriculture, where there is a vast scope for mycorrhizal technological application, it can be said that participation from the Indian scientific community was low.

# Commercial production of AMF through industrial mode and its large-scale application

#### Dr Alok Adholeya

Centre for Mycorrhizal Research, TERI (The Energy and Resources Institute), Darbari Seth Block, Habitat Place, Lodhi Road, New Delhi – 110 003, India

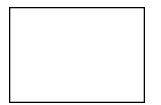
ICOM (International Conference on Mycorrhiza) IV recently held in Montreal, Canada, on 10–15 August 2003, was an important event for the scientific community working on a common subject with immense expertise to interact, communicate, and exchange their findings and impressions on the complex mycorrhizal symbiosis. Global acceptance exists on the role of mycorrhizal symbiosis in plantyield enhancement along with other positive benefits like disease resistance and stress tolerance, which were highlighted in the event. The need for its utilization as a biofertilizer is known among the scientists. The fungus is known to occur in soil universally but in fewer numbers, especially in tropical conditions. Dr Alok Adholeya, Senior Fellow and Area Convenor, Centre for Mycorrhizal Research, TERI, India, participated in this conference. Dr Adholeya has perfected the *in vitro* technology of cultivation of the AM fungi and has utilized this technique for mass production of AM biofertilizer. The importance of this *in vitro* technique can be gauged by the fact that there was a special session on this technique in ICOM-IV and Dr Adholeya was invited as a speaker.

Dr Adholeya delivered a talk on this technology that was developed and transferred to industries through which, for the first time, commercial production of mycorrhiza in India commenced. A detailed account on technology development – including identification of rate limiting factors, process parameter optimization, quality assessment bioassays, benchmarking, formulation, storage, and shelf life - was provided. The product has since reached the end users, that is, farmers. After the first cropping trial, the evaluation was also undertaken on product acceptability and assessment on its future potential. Data on the same was presented during the talk. A detailed account on multi-location field trials, which were undertaken under several agroecological zones was also presented. Several crops belonging to cereals, cash crops, vegetables, horticulture, and forestry plant species have shown improved yield on farmers' field due to inoculation with this commercial product. The yield improvement in agricultural crops was substantial in spite of the reduced phosphatic fertilizer application by up to 50%. This resulted in better cost-benefit ratio in addition to protection of soil from pollution due to excessive use of chemical

fertilizers. The talk was well received and praised for such an important achievement from a thirdworld country. There was high enthusiasm among the industry and researchers regarding the achievements highlighted.

The work carried out on the mass production technology was undertaken with the partial support received from the DBT (Department of Biotechnology), Government of India. Subsequent tripartite agreement was also signed by the recipient industries for technology transfer with the DBT and TERI. Commercial products, which are now available in the market from two industries, are being received well and farmers are drawing benefits from this new product.

In addition to his talk, Dr Adholeya participated actively in discussions in other sessions and in special lectures, and exchanged ideas with fellow scientists.



**Centre for Mycorrhizal Culture Collection** 

# Interactions between arbuscular mycorrhizal fungi and plant-growth promoting rhizobacteria

#### Reena Singh and Alok Adholeya

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

Adverse climatic conditions added with poor soil quality and small farm holdings are the major limitations for agriculture and other land-use activities in India. Our whole system of agriculture depends, in many important ways, on microbial activities and there appears to be a tremendous potential for making use of microorganisms in increasing crop production. There exists an immense diversity amongst microorganisms and even within a group of microorganisms. Microbial diversity encompasses a spectrum of microscopic organisms, including bacteria, fungi, actinomycetes, algae, and protozoa. The agriculturally important microorganisms have their own unique place and scope in the overall context of agrobiodiversity. They play an important role in nutrient acquisition for plants viz, N, P, K, and other microelements. Indirect influence on crop production can also be accrued by inoculation with biocontrol agents. However, in order to maximize beneficial effects of microbial activity, greater understanding of the factors influencing microbial diversity and activity in the soil is

needed. Studies on interactions of various organisms in the rhizosphere of legume host and the mechanisms involved would help evolve a method for improving the ecology and economics.

AMF (arbuscular mycorrhizal fungi) are an important component of sustainable agriculture. They can increase plant growth under low-fertility conditions and are of particular interest for wellfertilized agricultural soils. They can also improve tolerance towards different kinds of stress such as drought or resistance towards root pathogens. These fungi can enhance mineral nutrient acquisition in host plants. Phosphorus, nitrogen, zinc, and copper acquisition are most commonly reported elements being enhanced by AMF in plants, but the acquisition of other mineral nutrients required for plant growth may also be enhanced. It is well known that a considerable number of bacterial species are also able to exert a beneficial effect on plant growth. Mostly they are associated with the plant rhizosphere hence they are called rhizobacteria. This group of bacteria has been

termed plant-growth promoting rhizobacteria, and amongst it are strains from genera such as Alcaligenes, Acinetobacter Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium, and Serratia. They are used as biofertilizers or control agents for agricultural improvement, and there are numerous researchers for the area.

PGPR (plant-growth promoting rhizobacteria) include a range of organisms that live in close association with roots. PGPR are of great potential importance, and their culture and modes of action are the subject of active research. The possible mechanisms by which the PGPR aid plant growth include suppression of root pathogens through chelation of iron or production of antibiotics (Kloepper, Zablotowicz, Tipping et al. 1991), fixation of nitrogen (Chanway and Holl 1991), and production of plant hormones (Holl, Chanway, Turkington et al. 1988). PGPR are synergistic with mycorrhizae in stimulating plant growth (Chanway and Holl 1991), and may stimulate root colonization by mycorrhizal fungi (Meyer and Linderman 1986). There has been some success with PGPR in agriculture and commercial preparations are likely to become available (Linderman and Paulitz 1990).

It has been postulated that some PGPRs behave as mycorrhizal helper bacteria. It is likely that the phosphate solubilized by the bacteria could be taken up more efficiently by the plant through a mycorrhizal pipeline between roots and surrounding soil that allows nutrient translocation from soil to plant. Considerable evidence supports the specific role of phosphate solubilization in the enhancement of plant growth by phosphate-solubilizing microorganisms. However, not all laboratory or field trials have offered positive results. Therefore, the efficiency of the inoculation varies with the soil type, specific cultivars, and other parameters.

At the Centre for Mycorrhizal Research, TERI, experiments were conducted, both in the greenhouse and in fields, to test the interaction between efficient PGPRs and the AMF. The experiments involved five different cultivars of modern hexaploid wheat, five efficient AMF, and 11 PGPRs. Different parameters, which included yield, plant height, plant weight, and nutrient uptake (macronutrients viz. N, P, K, and micronutrients viz. Cu, Fe, Mo, Mn, Co) were analysed during the study and provided sufficient evidence that positive interaction exists between PGPRs and AMF. When they were supplied together to the plant, there was a curtailment of fertilizers' up to 50%.

#### References

Chanway C P and Holl F B. 1991

Biomass increase and associative nitrogen fixation of mycorrhizal *Pinus contorta* seedlings inoculated with a plant growth promoting *Bacillus* strain *Canadian Journal of Botany* 69: 507–511

Holl F B, Chanway C P, Turkington R, Radley R A. 1988

Response of crested wheatgrass Agropyron cristatum L. perennial ryegrass Lolium perenne and white clover Trifolium repens L. to inoculation with Bacillus polymyxa

Soil Biology and Biochemistry 20: 19–24

Kloepper J W, Zablotowicz R M, Tipping E M, Lifshitz R. 1991

Plant growth promotion mediated by bacterial rhizosphere colonizers, pp. 315-326

The Rhizosphere and Plant Growth, edited by D L Keister and P Cregan

[In Beltsville Symposia in Agricultural Research No. 14]

Linderman R G and Paulitz T C. 1990

Mycorrhizal rhizobacterial interactions, pp. 261–283

Biological Control of Soil Borne Plant Pathogens, edited by D Hornby

Meyer J R and Linderman R G. 1986

Response of sub-terranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant-growth promoting bacterium, *Pseudomonas putida* 

Soil Biology and Biochemistry 18:185–190

#### Pure mycorrhizal inoculum available

Spores of arbuscular mycorrhizal species *Glomus intraradices* produced through in vitro technology are available on request for molecular or other related research work.

The request may kindly be made to Dr Alok Adholeya, Senior Fellow and Area Convenor, Centre for Mycorrhizal Research, Bioresources and Biotechnology Division, TERI (The Energy and Resources Institute), Habitat Place, Lodhi Road, New Delhi – 110 003, India.

Tel (+91 11) 2468 2111/ 2468 2100 Fax (+91 11) 2468 2144/ 2468 2145 *E-mail* aloka@teri.res.in

# **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.

- Agriculture Ecosystems and Environment
- Applied Soil Ecology
- FEMS Microbiology Ecology
- Folia Geobotanica
- Journal of Arid Environments

- Mycorrhiza
- New Phytologist
- Plant and Soil
- Revista Brasilera de Ciencia Do Solo

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]		
Schloter M *, Dilly O, and Munch J. 2003	Indicators for evaluating soil quality Agriculture Ecosystems and Environment 98(1-3): 255–262 [*Schloter M, GSF, Natural Research Centre of Environment and Health, Institut of Soil and Ecology, Ingelstadter Landstr 1, D-85764 Neuherberg, Germany]		
Vivas A, Voros A, Biro B, Barea J M, RuizLozano J M, Azcon R*. 2003	Beneficial effects of indigenous Cd-tolerant and Cd-sensitive Glomus mosseae associated with a Cd-adapted strain of Brevibacillus sp. in im- proving plant tolerance to Cd contamination Applied Soil Ecology 24(2): 177–186 [*Azcon R, CSIC, Estac Expt Zaidin, Dept Microbio Suelo and Sistemas Simbioticos, Prof. Albareda 1, E-18008 Granada, Spain]		
Sood S G. 2003	Chemotactic response of plant growth promoting bacteria towards roots of vesicular arbuscular mycorrhizal tomato plants FEMS Microbiology Ecology 45(3): 219–227 [Sood SG, Savannah State University, Savannah, GA 31404, USA]		
Redecker D*, Hijri I, and Wiemken A. 2003	Molecular identification of arbuscular mycorrhizal fungi in roots: perspec- tives and problems Folia Geobotanica 38(2): 113–124 [*Redecker D, University of Basel, Institute of Botany, Hebelstr 1, CH-4056 Basel, Switzerland]		
Rydlova J* and Vosatka M. 2003	Effect of Glomus intraradices isolated from Pb-contaminated soil on Pb uptake by Agrostis capillaris is changed by its cultivation in a metal-free substrate Folia Geobotanica 38(2): 155–165 [*Rydlova J, Academy of Science Czech Republic, Institute of Botany, CS-25243 Pruhonice, Czech Republic]		
Sykorova Z,* Rydlova J, and Vosatka M. 2003	Establishment of mycorrhizal symbiosis in Gentiana verna Folia Geobotanica 38(2): 177–189 [*Sykorova Z, Academy of Science Czech Republic, Institute of Botany, CZ-25243 Pruhonice, Czech Republic]		
Vohnik M,* Lukancic S, Bahor E, Regvar M, Vosatka M, Vodnik D. 2003	Inoculation of Rhododendron cv. Belle-Heller with two strains of <i>Phialocephala fortinii</i> in two different substrates <i>Folia Geobotanica</i> 38(2): 191–200 [*Vohnik M, Acad Sci Czech Republic, Institute of Botany, CZ-25243 Pruhonice, Czech Republic]		

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]
Gryndler M,* Chvatalova I, Gryndlerova H, Hrselova H, Balaz M. 2003	Searchable database of soil- and mycorrhiza-related scientific reports (DSM) Folia Geobotanica 38(2): 238 [*Gryndler M, AS CR, Institute of Microbiology, Videnska 1083, CZ-14220, Prague 4, Czech Republic]
Bohrer G*, KaganZur V, RothBejerano N, Ward D, Beck G, Bonifacio E. 2003	Effects of different Kalahari-desert VA mycorrhizal communities on min- eral acquisition and depletion from the soil by host plants Journal of Arid Environments 55(2): 193–208 [*Bohrer G, Duke University, Department of Civil and Environment Engineering, Box 90287, Durham, NC 27708 USA]
Turnau K* and MesjaszPrzybylowicz J.	Arbuscular mycorrhiza of <i>Berkheya coddii</i> and other Ni-hyperaccumulating members of Asteraceae from ultramafic soils in South Africa <i>Mycorrhiza</i> 13(4): 185–190 [*Turnau K, Jagiellonian University, Institute of Botany, Ul Lubicz 46, PL-31512 Krakow, Poland]
Renker C, Heinrichs J, Kaldorf M, Buscot F.* 2003	Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field Mycorrhiza 13(4): 191–198 [*Buscot F, University of Jena, Department of Environmental Science, Institute of Ecology, Dornburger Str 159, D-07743 Jena, Germany]
Diedhiou P M, Hallmann J, Oerke E C,* Dehne H W. 2003	Effects of arbuscular mycorrhizal fungi and a non-pathogenic Fusarium oxysporum on Meloidogyne incognita infestation of tomato Mycorrhiza 13(4): 199–204 [*Oerke EC, Institute of Plant Dis, Nussallee 9, D-53115 Bonn, Germany]
MoreiraSouza M, Trufem S F B, GomesdaCosta S M, Cardoso E J B N.* 2003	Arbuscular mycorrhizal fungi associated with Araucaria angustifolia (Bert.) O. Ktze Mycorrhiza 13(4): 211–215 [*Cardoso EJBN, University of Sao Paulo, Dept Solos and Nutr Plantas, Caixa Postal 09, BR-13418900 Piracicaba, SP, Brazil]
Gebauer G* and Meyer M. 2003	N-15 and C-13 natural abundance of autotrophic and mycoheterotrophic or- chids provides insight into nitrogen and carbon gain from fungal association New Phytologist 160(1): 209–223 [*Gebauer G, University of Bayreuth, Lehrstuhl Pflanzenokol, POB 101251, D-95440 Bayreuth, Germany]
Spriggs A C, Stock W D, and Dakora F.* 2003	Influence of mycorrhizal associations on foliar delta N-15 values of legume and non-legume shrubs and trees in the fynbos of South Africa: implica- tions for estimating N-2 fixation using the N-15 natural abundance method <i>Plant and Soil</i> 255(2): 495–502 [*Dakora F D, Cape Technikon, Research and Development, POB 652, ZA-8000 Cape Town, South Africa]
Rodrigues L A,* Martins M A, and Salomao M S M B. 2003	Use of mycorrhizas and rhizobium in intercropping system of <i>Eucalyptus</i> and Sesbania. I - Growth, uptake and transfer of nitrogen between plants Revista Brasileira de Ciencia Do Solo 27(4): 583–591 [*Rodrigues LA, University Estadual Norte Fluminense, CCTA, Av Alberto Lamego 2000, Campos Goytacazes, BR-28013600 Campos Dos Goytacazes, RJ, Brazil]
Rodrigues L A,* Martins M A, and Salomao M S M B. 2003	Use of mycorrhizas and rhizobium in intercropping system of <i>Eucalyptus</i> and <i>Sesbania</i> . II - Phosphorus uptake and efficiency of use and phosphate-fractions <i>Revista Brasileira de Ciencia Do Solo</i> 27(4): 593–599 [*Rodrigues LA, University Estadual Norte Fluminense, CCTA, Av Alberto Lamego 2000, Campos Goytacazes, BR-28013600 Campos Dos Goytacazes, RJ, Brazil]

# Forthcoming events

Conferences, congresses, seminars, symposiums, and workshops

Tropical (CIAT), Cali, <b>Colombia</b> 8-14 March 2004	Sixth International Scientific Meeting of the Cassava Biotechnology Network (CBN) A.A. 6713, Cali, Colombia
	Fax +57 2 4450073 (direct), +1 650 8336626 (via USA) Tel. +57 2 4450000 (direct), +1 650 8336625 (via USA) E-mail ciat@cgiar.org Web site www.ciat.cgiar.org
Mexico City, <b>Mexico</b> 11-14 March 2004	<b>46th Annual Maize Genetics Conference</b> MU Conference Office, University of Missouri-Columbia, MU Extension, 344 Hearnes Center, Columbia, Missouri 65211
	Fax (1) +573 882 1953 Tel. (1) + 573 882 4087 E-mail muconf4@missouri.edu
Salt Lake City, <b>Utah</b> 4–8 April 2004	NIAA Annual Meeting: farmland Security—ensuring our future National Institute for Agriculture, 1910 Lyda Avenue Bowing Green, KY 42104-5809
	Fax 270 782 0188 Web site http://animalagriculture.org/annual_meeting/2004
Hinxton, Cambridge, <b>UK</b> 14–17 April 2004	<b>Genomes 2004: International Conference on the Analysis of</b> <b>Microbial and Other Genomes</b> TIGR Conference Department, 9712 Medical Center Drive, Rockville, Maryland 20850
	Fax 301 838 0229 Tel. 301 795 7964 E-mail registration@tigr.org
Rhodes Island, <b>Greece</b> 29 April–4 May 2004	Seed Ecology 2004: An International Meeting on Seeds and the Environment Rodos Palace Hotel, Rhodes Island, Greece, Costas A Thanos
	Fax 30 210 7274656 E-mail cthanos@biol.uoa.gr
Berlin, <b>Germany</b> 24–26 May 2004	<b>ISF World Seed Congress 2004</b> Mr Joël Diaz, ISF (International Seed Federation) Chemin du Reposoir 7, 1260 Nyon, Switzerland
	<i>Tel.</i> +41 22 365 4425 <i>Fax</i> +41 22 365 4421 <i>E-mail</i> register@worldseed.org <i>Web site</i> www.worldseed2004.com

Editor Alok Adholeya • Associate Editor Shantanu Ganguly • Assistant Editor Pritika Kalra