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About TERI

A dynamic and flexible organization with a global vision and a local focus, TERI (The Energy and Resources Institute, formerly known as the Tata Energy Research Institute) was established in 1974. TERI's focus is on research in the fields of energy, environment, and sustainable development, and on documentation and information dissemination. The genesis of these activities lie 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.

Biotechnology and Management of Bioresources Division

Focusing on ecological, environmental, and food security issues, the activities of the Biotechnology and Management of Bioresources Division include working with a wide variety of living organisms, sophisticated genetic engineering techniques, and, at the grass-roots level, with village communities.

Mycorrhiza Network

The Biotechnology and Management of Bioresources Division's Mycorrhiza Network works through its three wings—the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhizal Culture Collection), and *Mycorrhiza News*.

The MIC is primarily responsible for establishing databases on Asian mycorrhizologists and on mycorrhizal literature (RIZA), which allows information retrieval and supplies documents on request. The CMCC has the main objectives of procuring strains of both ecto and vesicular arbuscular mycorrhizal fungi from India and abroad; multiplying and maintaining these fungi in pure cultures; screening, isolating, identifying, multiplying, and maintaining native mycorrhizal fungi; developing a database on cultures maintained; and providing starter cultures on request. Cultures are available on an exchange basis or on specific requests at nominal costs for spore extraction or handling.

Mycorrhiza News

Mycorrhiza News – a quarterly newsletter publishing articles compiled from RIZA – invites short papers from budding and senior mycorrhizologists, giving methodologies being used in mycorrhizal research, providing information on forthcoming events on mycorrhiza and related subjects, listing important research references published during the quarter, and highlighting the activities of the CMCC.

Contents

Nitrogen assimilation in mycorrhizal fungi/roots

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Plant roots can take up nitrogen from the soil both in the form of nitrate nitrogen or ammonium nitrogen. In nitrate assimilation, NO_2^- (nitrate) is first reduced to nitrite by the cytoplasmic enzyme, nitrate reductase. Nitrite is then reduced to ammonia by nitrite reductase activity. An amino group from ammonia is added to amino acid glutamate to give the amide glutamine. This reaction is catalysed by the enzyme GS (glutamine synthetase). Glutamine produces two molecules of glutamate by the enzyme glutamate synthetase or GOGAT (glutamine-oxoglutarate, aminotransferase). This is the GS/GOGAT pathway of ammonium assimilation. Another pathway of ammonium assimilation is the GDH (glutamate dehydrogenase) pathway. While the GS/GPGAT pathway is now firmly established in higher plant roots, nitrogen assimilation in several ectomycorrhizal fungi occurs prominently through the GDH pathway (Botton and Dell 1992). The assimilation of nitrogen by mycorrhizal fungi/roots is more or less on the same pattern as assimilation by plant roots. Mycorrhizal fungi are known to produce both nitrate reductase enzymes and ammonium assimilating enzymes.

Nitrate reductase activity in mycorrhizal fungi

Nitrate reductase activity in pure culture of ectomycorrhizal fungi

Studies conducted at the INRA (Laboratoire Institut Nationale de la Recherche Agronomique) de Recherches sur les Symbiotes des Racines, 9 Place Viala, Montpellier, Cedex, France, showed that the growth of *Hebeloma cylindrosporum* on nitrate medium was associated with nitrate reductase activity equivalent to that measured in herbaceous angiosperms. Nitrate was not indispensable for the induction of nitrate reductase, as thalli cultivated on ammonium had the same nitrate reductase activity as thalli cultured on nitrate. However, the simultaneous presence in the culture medium of ammonium with an organic acid such as malate, citrate, or succinate, caused a diminution of nitrate reductase activity. Glutamine or asparagine by-products in the assimilation of ammonium and malate could intervene in the regulation of this enzyme. The data suggests that the absence of nitrogen or the presence of a nitrogen source such as ammonium, inappropriate for growth, derepressed nitrate reductase in this fungus (Scheromm, Plassard, and Salsac 1990).

Other studies conducted at the above laboratory showed that the growth of ectomycorrhizal isolates on ammonium and nitrate was highly variable as a function of the species. There was a significant correlation between the nitrate reductase activities and the growth rate on nitrate. However, this correlation was not significant with homokaryons of *Hebeloma cylindrosporum*. These homokaryons also had very different abilities to use nitrium phytate; in particular, the phytase activities measured in the culture media of these isolates were highly variable (Plassard, Scheromm, Tillard, *et al.* 1987).

In further studies conducted at the above laboratory, in-vitro measurement of nitrogen reductase activity was first performed on the

colourless ectomycorrhizal fungus, *Hebeloma cylindrosporum*. In order to extend this assay to other species, which can be coloured, composition of extraction buffer was tested by mixing tissues from *H. cylindrosporum* and from species such as *Suillus collinitus*, *S. bellini*, *S. bovinus*, *Pisolithus tinctorius,* and *Cenococcum geophilum*. The addition of PVP (polyvinylpyrolidone) in the buffer made it possible to measure nitrate reductase activity of *P. tinctorius*. The capacity of ectomycorrhizal fungi for nitrate assimilation can be used for a selection of effective fungal isolates for their rational use. The research on nitrogen reductase activity should, therefore, elucidate the role of this enzyme in the nitrogen metabolism of ectomycorrhizal symbionts and establish criteria for selecting effective strains (Plassard, Lerandy, Saint Jorre, *et al.* 1990).

In studies conducted at the Finnish Forest Research Institute, Parkano Research Station, Parkano, Finland, the nitrate reducing capacity of pure cultures of *Cenococcum geophilum*, *Paxillus involutus* (strains 1 and 2), *Piloderma croceum*, *Suillus variegatus* (strains 1 and 2), and an ectendomycorrhizal (E-strain) fungus was measured using an in vivo nitrate reductase (EC 1.6.6.3) assay. Differences in nitrate reductase activities were observed between species and strains. The nitrate concentration of the culture medium influenced the nitrate reductase activities of the E-strain fungus and one strain of S. *variegatus*. The nitrate concentration reductase activity of certain species and strains was a function of nitrate. Addition of ammonium to the growth medium did not have any significant effect on the in-vivo or in-vitro nitrate reductase activity. The in-vivo nitrate reductase activity in the mycelia of *C. geophilum* and the E-strain fungus decreased during 28 days growth in the modified Melin-Norkrans medium. For mycelia of *P. involutus*, *P. croceum,* and *S. variegatus* grown on agar, the in-vitro assays showed higher nitrate reductase activity than the in-vivo assays (Sarjala 1990).

In studies conducted at the University of Saskatchewan, Department of Biology, Saskatoon, Canada, the regulation of nitrate reductase (EC 1.6.6.3) in the mycorrhizal ascomycete, *Wilcoxina mikolae* var. *mikolae*, was studied with the aim of improving understanding of nitrate assimilation in mycorrhiza. It was possible to study induction, derepression, and repression of nitrate reductase individually and in combination by utilizing urea, the neutral nitrogen source. The nitrate reductase activity required specific induction by nitrate and was not present in derepressed urea grown cultures. The induction of nitrate reductase by nitrate required de-novo protein synthesis as it was inhibited by cycloheximide and actinomycin D. Both ammonium and glutamine in the medium repressed the nitrate reductase activity. The repression by ammonium appeared to require its assimilation since treatment of mycelia with L-methionine-DL-sulfoximine, a specific inhibitor of glutamine synthetase, reduced ammonium repression. These studies demonstrated that the regulation of nitrate reductase in Wilcoxina is unlike the constitutive and non-repressible nitrate reductase reported in mycorrhizal basidiomycetes (Prabhu, Wilcox, Boyer 1996).

In studies conducted at the Department of Biology, State University of New York, College of Environmental Science and Forestry, Syracuse, New York, USA, the strategy adopted for the extraction of nitrate reductase from *Wilcoxina mikolae* var mikolae included the development of culture conditions that were amenable to depression and induction of nitrate reductase as well as consideration of the liability of the enzyme. The growth on neutral nitrogen source, the use of an artificial electron donor, and rapid partial purification in the presence of PMSF (phenyl sulfonyl fluoride) contributed to the extraction of a fully functional NADPH (nicotinamide adenine dinucleotide phosphate)-specific nitrate reductase from Wilcoxina. The optimum pH for nitrate reduction by this enzyme was 7.0 in 100 mM phosphate. The apparent Km (Michaelis-Menten constant) for NADPH was 35 microM and the Kd for nitrate was 200 microM. The functional properties of this enzyme were similar to those reported for other filamentous fungi. The nitrogen reductase from Wilcoxina is thought to be highly prone to nitrogen metabolite repression and inactivation by endogenous factors during extraction (Prabhu, Wilcox, and Boyer 1995).

In studies conducted by the Mycorrhiza Research Group, School of Science, University of Western Sydney, Kingswood, New South Wales, Australia, *Pisolithus tinctorius, Tylospora fibrillosa,* and a mycosymbiont isolated from *Pisonia grandis* were compared with regard to their relative abilities to produce key enzymes of inorganic nitrogen assimilation. Nitrate reductase activities in *P. grandis* symbiont and *T. fibrillosa* were significantly lower than in *P. tinctorius*. Nitrate reductase activity was expressed in all three fungi regardless of the nitrogen source in the medium, but it diminished in *P. tinctorius* following continued exposure to either $NO3^{-}$, NH4+, glutamine, or $NO3^{-}$ + glutamine (Sharples and Cairney 1998).

Nitrate reductase activity in ecto-mycorrhizal roots

In studies conducted at the Finnish Forest Research Institute, Parkano Research Station, Parkano, Finland, nitrate reductase activity in seedlings of Scots pine (*Pinus sylvestris*) inoculated with *Cenococcum geophilum*,

Paxillus involutus, *Piloderma croceum,* and *Suillus variegatus* was measured. This study was conducted to investigate the effects of symbiosis and nitrate nutrition on nitrate assimilation of seedlings, and on effects of selecting different fungus species as symbionts on assimilation. Nitrate reductase activity was greater in mycorrhizal pine roots than was previously found in fungus symbiont alone, but less than in roots of non-mycorrhizal pine seedlings. Differences between fungus species, previously observed in pure culture conditions, were not seen in this study for mycorrhiza synthesized with the same fungus species. Increase in nitrate concentration of the nutrient solution increased the proportion of nitrate reductase activity in needles. Mycorrhizal root tips had higher nitrate reductase activity than non-mycorrhizal root tips in the same roots system (Sarjala 1991).

Nitrate reductase activity in vesicular-arbuscular mycorrhizal fungi

In studies conducted at the Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India, extramatrical chlamydospores of *Glomus fasciculatum*, *G. mosseae*, *G. intraradices*, *G. caledonium*, *Gigaspora margarita*, *G. calospora*, *Endogone discii,* and *Acaulospora* sp. collected from single species pot cultures maintained with *Cenchrus ciliaris* were found to possess the ability to reduce nitrate to an extent varying from 1.5–3.8 micromoles per tube of 200 extramatrical chlamyolospores per 24 hours (Tilak and Dwivedi 1990).

Studies conducted at the Department of Botany, Jawahar Lal Nehru University, Jodhpur, India, on plant samples from 48 days old seedlings of *Ziziphus mauritiana* showed that GS activity was higher in all the treatments as compared to nitrate reductase activity. Addition of VAM (vesicular arbuscular mycorrhiza) increased the activities of both these enzymes. However, different VAM species varied in their efficacy to increase these enzymatic activities. Among the five VAM species used in the experiment, *Glomus fasciculatum* was found to be the most efficient for *Z. mauritiana,* as it increased most effectively the activities of GS and NR (nitrate reductase) in this multipurpose fruit tree of the Indian Thar Desert (Mathur, Nishi, and Vyas 1995).

Genetic evidence of nitrate reductase in VAM and other fungi

Studies conducted at the Botanisches Institut, Universitat Koin, Gyrhofstrasse 15, Kolin, Germany, showed that by using primers synthesized from two conserved regions and employing polymerase chain reaction, a DNA segment coding for part of the apoprotein of assimilatory nitrate reductase could be amplified from the fungi *Aspergillus nidulans*, *Pythium intermedium*, *Phytophthora infestans*, *Phytophtora megasperma,* and *Glomus* sp. D13. Sequencing of the amplificates as well as DNA hybridization revealed strong homologies with the nitrate reductase gene in all cases. The digoxigenin-labelled amplificate from *Glomus* sp. hybridized with DNA isolated from Glomus spores. The data from these gene-probing experiments are generally in accord with the published results from enzyme measurements. Thus, assimilatory nitrate reductase occurs in saprophytic, parasitic, as well as AMF (arbuscular mycorrhizal fungi). No amplificates with these primers were obtained with DNA isolated from *Mucor mucedo* and *Saprolegnia ferax*. Such results agree with the failure to detect nitrate assimilation physiologically in these two organisms (Kaldorf, Zimmer, and Bothe 1994).

Effect of drought on nitrate reductase activity

In the studies conducted at the Departmento de Microbiologia del Suelo y Sistemas Simbioticos, Estacion Experimental del Zaiden (CSIS), Granada, Spain, the effect of VAM fungus, *Glomus fasciculatum*, on growth and nitrogen form assimilation was measured on onion (*Allium cepa*) grown under well-watered (–0.04 MPa [megapascals]) or drought conditions (–0.17 MPa) with two uninoculated controls (one provided with phosphate). These three treatments were supplemented with 2.0 mM nitrogen as nitrate and ammonium in a 1:1 ratio. Nitrate reductase and glutamine synthetase activities in shoot and root tissue were determined when water was maintained at –0.04 MPa or –0.17 MPa in the growth medium. The most marked increasing effect of VAM colonization on nitrate reductase activity was in root tissue. Under water limitations, the effectiveness of *G. fasciculatum* increasing nitrate reductase activity in plants was enhanced. The proportion of nitrate assimilation into root was increased in VAM plants, particularly under well-watered conditions. Mycorrhizal plants reached a high specific and total GS activity in shoots and roots than phosphorus fertilized plants at 0.04 MPa. The mycorrhizal effect on GS activity under water stress (-0.17 MPa) was evident only in roots being compared to those found in phosphorus fertilized plants. The proportion of GS in roots was increased in VAM plants under whatever soil water conditions. The results are evidence of direct effect on absorption, translocation, and assimilation of both nitrogen forms by the endomycorrhizal system. Mycorrhizal plants can utilize nitrate form more efficiently than ammonium form (Azcon and Tobar 1998).

Another study conducted at the above institute on lettuce, either inoculated with VAM fungi, *Glomus deserticola*, *G. fasciculatum*, or *G. mosseae,* or uninoculated with or without phosphorus fertilization and grown under controlled conditions at constant soil water potential (-0.04 MPa or -0.17 MPa) during the last six weeks of plant growth, showed that mycorrhizal plants had higher nitrate reductase activity than uninoculated plants, particularly under water stress conditions. Control plants had 57% less nitrate reductase activity than *G. deserticola* colonized plants under well-watered conditions, with a reduction in nitrate reductase activity of 79% when the plants were subjected to drought stress. Under well-watered conditions, the phoshphous-fertilized plants had similar or higher growth and phosphorus content than the *G. mosseae* and *G. fasciculatum* mycorrhizal plants, with lower nitrate reductase activity in phosphorus-fertilized than in VAM plants. It is suggested that either the VAM fungi increased the nitrate reductase activity in the host plant (regardless of the phosphorus content) or that the VAM fungi have enzymatic activity per se. Under the experimental conditions, plants colonized by different VAM fungi showed different nitrate reductase activities. Drought stress decreased nitrate reductase activity, but the decrease was less in mycorrhizal than in uninoculated plants. This effect may be a factor in the drought tolerance of mycorrhizal plants (Ruiz-Lozano and Azon 1996).

Intraspecific variation in nitrogen reductase activity

In studies conducted at the Universite Claude Bernard, Lyon 1, URA CNRS 697 Ecologie Microbienne, Villeurbanne, Cedex, France, intraspecific variability in the activity of NR was studied in *Hebeloma cylindrosporum* at the interstrain and intrastrain levels, the latter within a population of 11 wild dikaryotic strains collected from four locations less than 100 km away from one another. The NR activity of wild strains ranged from 201–700 nmol NO_2 synthesized per hour mg fungal protein whilst that of 20 sibmonokaryons (5 per mating type) of the HCl strain varied from 51–510 nmol NO_2 synthesized per hour mg fungal protein. Fifty controlled dikaryotic mycelia obtained from all the compatible fusions had NR activity variation of the same order of magnitude as that recorded at the interstrain level (72–689 nmol NO_2 per hour mg fungal protein). The variation of NR activity in these controlled dikaryons demonstrated that the additive component of this variation accounted for less than 1% of the total observed variation. The NR activity of any one controlled dikaryon could not, therefore, be predicted from the activity of its parental monokaryons. However, 14 of the 50 controlled dikaryons exhibited NR activity higher than that of the HCl parental dikaryon (Wagner, Gay, and Debaud 1989).

Production of ammonium assimilating enzymes by mycorrhizal fungi

In studies conducted at the Research Station, Agriculture Canada, Saint-foy, Quebec, Canada, on Jack pine seedlings, inoculated or not with *Laccaria bicolor* or *Pisolithus tinctorius* and grown for 16 weeks in growth chambers, five enzymes involved with nitrogen assimilation were measured in the root systems as well as in pure cultures of mycorrhizal fungi. *P. tinctorius* in pure culture had no detectable activity of NR, GDH, GDCO (glutamate decarboxylase), or GOGAT, but did have some GS activity. *L. bicolor* in pure culture had no NR activity, low GDCO activity, and high GDS, GS, and GOGAT activity. The high levels of enzymatic activity present in *L. bicolor* indicate that it may play a greater role in the nitrogen metabolism of its host plant than *P. tinctorius*. ECM (ectomycorrhizal) infection clearly altered the enzyme activity in Jack pine roots, but the changes depended on the fungal associate. Non-ectomycorrhizal root systems had higher NR, GS, GDH, and GDCO activities than ECM root systems. Root systems infected with *L. bicolor* had significantly greater NR and GDCO activity than those infected with *P. tinctorius*. Differences in the GS activity of the two fungi in pure culture corresponded to the GS activity of infected Jack pine roots (Vezina, Margolis, Mcafee, *et al.* 1989).

In studies conducted at the School of Biological Science, Murdoch University, Murdoch, Western Australia, the ammonia assimilation enzyme, glutanase dehydrogenase, was studied in extracts of spruce (*Picea excelsa*) roots, mycelium of mycorrhizal fungus (*Hebeloma* sp.), and associated mycorrhizae. Evidence from enzyme reactions in crude extracts, electrophoretic patterns and immunological tests using antibodies raised against purified NADP (nicotinamide adenine dinucleotide phosphate)-GDH of *Cenococcum geophilum* consistently showed that Hebeloma NADP-dependent GDH was active in spruce ectomycorrhizae. Histochemical studies associated same NADP-GDH activity with the Harting net. By contrast, the NADP-GDH fungal pathway was strongly suppressed in beech (*Fagus sylvatica*) associations with *Hebeloma crustuliniforme* and *Paxillus involutus* (Dell, Botton, Martin, *et al.* 1989).

In studies conducted at the Universite de Nancy I, Laboratoire de Biologie Vegetale et Forestiere, Vandoeuvre-les-Nancy, Cedex, France, NAD (nicotinamide adenine dinucleotide) and NADP dependant GDH (GDH and AAT [asparatate

aminotranferase]) were analysed in the ectomycorrhizal fungi in pure culture, in the mycorrhizae and in the non-mycorrhizal host roots of the association. The mycorrhizal associations studied were *Picea excelsa* (*P. abies)-Hebeloma* sp., *Pseudotsuga douglasii* (*P. menziesii*) *-Laccaria laccata*, *Fagus sylvatica-Hebeloma crustuliniforme,* and *F. sylvatica-Paxillus* involutus. NADP-GDH present in the fungus and NAD-GDH present in non-infected roots were both detected in *P. abies* and *P. menziesii* mycorrhizae. By contrast, fungal NAPD-GDH was suppressed in mycorrhizae of *F. sylvatica*. In the 4 associations, AAT found in the mycorrhizae corresponded to the host root isoforms; the fungal isoform was strongly repressed. Dissection of mycorrhizal tissues in spruce confirmed these results. The vascular cylinder full of fungus and cortical region including root cells and fungal hyphae revealed identical isoforms while no activity was found in the peripheral mycelium layer. According to the enzyme and the type of association investigated, isoenzymes found in mycorrhizae were variable (Chalot, Dell, Botton *et al.* 1990).

Further studies conducted at the above university showed that non-mycorrhizal roots of four *Eucalyptus* spp. (*E. diversicolor*, *E. globulus*, *E. nitens*, *E. regnans*) were characterized by a high activity of NAD-GDH, but only NADP-GDH was found in *Hebeloma westraliense* in pure culture. AAT, NAD-GDH, and NADP-GDH were investigated by enzyme assays and electrophoretic patterns on polyacrylamide gel. In association of these eucalypts with *H. westraliense*, both NAD and NADP-GDHs were detected. By contrast, NAD and NADP-GDHs found in the free living mycelia of *Laccaria laccata* were not detected in the associated ectomycorrhizae. In ectomycorrhizae of five species of eucalypts (*E. diversicolor*, *E. globulus*, *E. grandis*, *E. regnans,* and *E. urophylla*) associated with *Laccaria laccata*, *Scleroderma verrucosum,* or *Pisolithus tinctorius*, and also in ectomycorrhizae of Norway spruce and Douglas fir, fungal AAT, which was very active in free-living mycelia, was not detected in the symbiotic tissues. In contrast, the two root AAT isoenzymes remained active in the mycorrhizae and were even stimulated. The studies indicate that the fungal gene expression is moderated by the host plant but also depends upon the fungal associates. In another study, the activity and amount of fungal NADP-GDH polypeptide was strongly suppressed in beech ectomycorrhizae, but this enzyme remained fully active in Norway spruce or Douglas fir ectomycorrhiza (Botton and Dell 1992, 1994).

Studies conducted by the Mycorrhiza Research Group at the School of Science, University of Western Sydney, Kingswood, Australia, on *Pisolithus tinctorius*, *Tylospora fibrillosa,* and a mycosymbiont isolated from *Pisonia grandis* showed that specific activities of GDH were higher in *P. tinctorius* than in the other two fungi following NH4+ pre-treatment. GS activity did not differ significantly among the three fungi. In all three fungi, specific activities for GS were significantly higher than for GDH (Sharples and Cairney 1998).

Studies conducted at the Department of Botany, University of Toronto, Toronto, Ontorio, Canada, on *L. bicolor* grown axenically as a suspension of fine hyphae in defined buffered media and supplied with various carbon and nitrogen sources indicated that *L. bicolor* has the potential to assimilate ammonium by the activities of glutamine synthetase, NADH-GDH and, NADPH-GDH. It also contains active aspartate and AAT. The activities of GS, NADPH-GDH and AAT were greater in the presence of nitrate than in the presence of ammonium and declined as the culture aged, suggesting a biosynthetic role for these enzymes. In contrast, the activities of NADH-GDH and alanine aminotransferase increased during post-exponential growth, and also in cultures growing on amino acids as a carbon source, suggesting a catabolic role for these enzymes (Ahmad, Carleton, Malloch, *et al.* 1990).

Characterization of nitrogen assimilating enzymes

Glutamate dehydrogenase and Glutamine synthetase enzymes

In studies conducted at the Laboratory Microbiologie Forestiere, INRA-CNRF, Champenoux, Seichamps, France, the NADP-specific glutamate dehydrogenase (L-glutamate: NADP + oxido-reductase, EC 1.4.1.4) of the ectomycorrhizal ascomycete, *Cenococcum graniforme*, was purified two-fold to electrophoretic homogeneity. The native enzyme was shown to have a molecular weight of 320 000 and to be composed of six identical subunits with a molecular weight of 48 000. The pH optimum for the aminating reaction was 7.6 .cntdot. NADP-GDH showed a negative co-operativity with respect to ammonia (Km 1:2 mM, Km 2:8 mM). The Km values for alpha-ketoglutarate and NADPH were 2 mM and 0.03 mM, respectively. The physical and kinetics properties of this enzyme are similar with those reported for NADP-GDH of other fungi. Cross-reactivity of a rabbit monospecific antiserum raised against the NADP-GDH from *Sphaerpstilbe repens*, a saprophytic ascomycete, was tested against the enzyme of *C. graniforme*. The immunochemical homology of both enzymes was low, suggesting that a substitution occurs in the amino acid residue of the protein (Martin, Mastef, and Botton 1983).

In studies conducted at the University de Nancy I, Laboratoire, de Biology Vegetale et Forestiere, Vandoeuvra-les-Nancy, Cedex , France, GS and

NADP-dependent GDH, which play a key role in nitrogen assimilation in the ectomycorrhizal fungus, *Laccaria laccata*, were purified to apparent electrophoretic homogeneity by a three-step procedure involving DEAE (diethylaminoethyl) trisacryl and affinity chromatography, and DEAE-5PW fast protein liquid chromatography. This purification scheme resulted in a 23% and 62% recovery of the initial activity for GS and NADP-GDH, respectively. Purified GS had a specific activity of 713 nanomoles per second per milligram protein and a pH optimum of 7.2. Michaelis constants (mM) for the substrates were NH4+ (0.024), glutamate (3.2), glutamine (30), ATP (adenine triphosphate) (0.18), and ADP (adenine diphosphate) (0.002). The molecular weight (MW) of native GS was approximately 380 000; it was composed of eight identical subunits of Mr 42 000. Purified NADP-GDH had a specific activity of 430 nanomoles per second per milligram protein and a pH optimum of 7.2 (amination reaction). Michaelis constants (mM) for the substrates were NH4+ (5), 2-oxoglutarate (1), glutamate (26), NADPH (0.01), and NADP (0.03). Native NADP-GDH was a hexamer with a Mr of about 298 000 composed of identical subunits with Mr 47 000. Polyclonal antibodies were produced against purified GS and NADP-GDH. Immunoprecipitation tests and immunoblot analysis showed the high reactivity and specificity of the immune sera against the purified enzymes (Brun, Chalot, Botton, *et al.* 1995).

In further studies conducted at the above university, the NAD-dependent GDH (EC 1.4.1.2) from *Laccaria bicolor* was purified 410-fold to apparent electrophoretic homogeneity with a 40% recovery through a three-step procedure involving ammonium sulfate precipitation, anion-exchange chromatography on DEAE-trisacryl, and gel filtration. The molecular weight of the native enzyme determined by gel filtration was 470 kDa, whereas sodium dodecyl sulfate-polyacrylamide gel electrophoresis gave rise to a single band of 116 kDa, suggesting that the enzyme is composed of four identical subunits. The enzyme was specific for NAD(H). The pH optima were 7.4 and 8.8 for the amination and deamination reactions, respectively. The enzyme was found to be highly unstable, with virtually no activity after 20 days at –75 °C, 4 days at 4 °C, and after 1 hour at 50 °C. The addition of ammonium sulfate improved greatly the stability of the enzyme and full activity was still observed after several months at –75 °C. NAD-GDH activity was stimulated by Ca2+ and Mg2+ but strongly inhibited by Cu2+ and slightly by the nucleotides AMP (adenine monophosphate), ADP, and ATP. The Michaelis constants for NAD, NADH, 2-oxoglutarate, and ammonium were 282 microM, 89 microM, 1.35 mM, and 37 mM, respectively. The enzyme had a negative cooperativity for glutamate (Hill number of 0.3), and its Km value increased from 0.24 to 3.6 mM when the glutamate concentration exceeded 1 mM. These affinity constants of the substrates, compared with those of the NADP-GDH of the fungus, suggest that the NAD-GDH is mainly involved in the catabolism of glutamate, while the NADP-GDH is involved in the catalysis of this amino acid (Garnier, Berredjem, and Botton 1997).

Studies conducted at the University of Bielefeld, Faculty of Biology, Lehrstuhl Stoffwechselphysiol, Bielefeld, Germany, on axenic mycelia of *Suillus bovinus* grown in liquid media under continuous aeration with compressed air at 25 °C in darkness and with glucose as the only source of carbohydrate showed that in crude extracts of cells from NH4+-cultures, NADH-dependent GDH exhibited high aminating (688 nmol per mg protein per minute), and low deaminating (21 nmol per mg protein per minute) activities. Its Km values for 2-oxoglutarate and for glutamate were 1.43 mM and 23.99 mM, respectively. pH-optimum for amination was about 7.2, and that for deamination was about 9.3. GS activity was comparatively low (59 nmol per mg protein per minute). Its affinity for glutamate was poor (Km = 23.7 mM), while that for the NH4+ replacing NH_2OH was high (Km = 0.19 mM). pH optimum was found at 7.0. GS (= GOGAT) revealed similar low activity (62 nmol per mg protein per minute), Km-values for glutamine and for 2-oxoglutarate of 2.82 mM and 0.28 mM, respectively, and pH- optimum around 8.0. Aspartate transaminase (= GOT) exhibited similar affinities for aspartate (Km = 2.55 mM) and for glutamate (Km = 3.13 mM), but clearly different Km-values for 2-oxoglutarate (1.46 mM) and for oxaloacetate (0.13 mM). Activity at optimum pH of about 8 was 506 nmol per mg protein per minute for aspartate conversion, but only 39 nmol per mg protein per minute at optimum pH of about 7 for glutamate conversion. Activity (599 nmol/mg protein/minute), substrate affinities (Km for alanine = 6.30 mM for 2-oxoglutarate = 0.45 mM) and pH-optimum (6.5–7.5) proved alanine ransaminase (= GPT) also important in distribution of intracellular nitrogen. There was comparatively low activity of obviously constitutive enzyme, urease (42 nmol per mg protein per minute), whose substrate affinity was rather high (Km = 0.56 mM) (Grotjohann, Kowallik, Huang *et al.* 2000).

Aspartate amino transferase enzymes

In studies conducted at the Laboratoire de Physiologie Vegetale, Universite de Nancy, Vandoeuvre-les-Nancy, Cedex, France, the aspartate aminotransferase (EC 2.6.1.1) of *C. geophilum* was purified to electrophoretic

homogeneity by a four step procedure including ammonium sulfate fractionation, hydrophobic and anion exchange chromatography, and, finally, gel filtration. The enzyme had a molecular mass estimated to be 155 kDa and was composed of four identical subunits of C40 kDa. The optimum pH values for the formation of glutamate and its utilization were 7.6 and 6.2, respectively. The enzyme remained fully active at 4 °C for at least 10 days, and even for six weeks in the presence of alpha-ketoglutarate. A kinetic study at different aspartate and alpha-ketoglutarate concentration indicated that the aminotransferase operates through a Ping-Pong Bi-Bi reaction mechanism. The Km values for aspartate, alpha-ketoglutarate, glutamate, and oxaloacetate were, respectively, 0.46, 0.34, 3.4, and 0.048 mM. The purified enzyme showed a specific requirement for pyridoxal phosphate. In addition to aspartate, the protein could transaminate asparagine, glycine, and, to a lesser extent, tyrosine, phenylalanine, tryptophan, methionine, and threonine when alpha-ketoglutarate was used as amino acceptor substrate (Khalid, Boukroute, Botton, e*t al.* 1988).

Cellular localization of nitrogen assimilating enzymes

In studies conducted at the Department of Microbial Ecology, University of Lund, Ecology Building, Lund, Sweden, cellular localization of GS and NADP-GDH in *Laccaria laccata* using an immunogold technique, combined with transmission electron microscopy, was examined using antibodies raised against two enzymes (purified from *L. laccata*) in rabbits. This technique clearly demonstrated cytoplasmic localization of GS and NADP-GDH. In addition, GS was also detected in dense cytoplasmic patches. The average numbers of gold particles indicate a higher amount of GS polypeptide compared with that of NADP-GDH polypeptide in nitrate-grown cells. These observations also correlated with estimates calculated from purified enzyme preparations of nitrate-grown mycelia. For determining the localization of these enzymes in ectomycorrhizal roots, thin sections of Douglas-fir/*L. laccata* ectomycorrhizal roots were first treated either with anti-GS or anti-NADP-GDH specific antibody, and then with colloidal gold marker. The gold label for NADP-GDH or GS was always distributed over the entire section of the ectomycorrhizae where fungal cells occurred. The distribution of nitrogen-assimilating enzymes among different tissues did not differ significantly. No labelling was observed in host cells (Brun, Chalot, and Botton 1993; Brun, Chalot, Duponnois, *et al.* 1994).

Ammonium assimilation pathway in ectomycorrhizal fungi

While the GS/GOGAT pathway is firmly established in higher plants, nitrogen assimilation in several ectomycorrhizal fungi occurs prominently through the GDH pathway (Botton and Dell 1992). Studies conducted at the Department of Biology, University College, London, UK however showed that glutamine was the major product accumulated following transfer of nitrogen-limited cultures of *P. tinctorius* to an ammonium medium. Experiments in which mycelium was transferred to 15NH4+ showed that glutamine amide was the most heavily labelled product. Assimilation of ammonium into glutamate was inhibited by azaserine. It is concluded that the kinetics of 15N-labelling and the effects of azaserine and methionine sulfoximine on the distribution of 15N-labelled products are consistent with the operation of the glutamate synthase cycle. No evidence was found for ammonium assimilation via GDH. From the labelling pattern observed in mycelium treated with aminooxyacetate, it is suggested that transamination reactions are an important source of glutamate for the synthesis of glutamine (Kershaw and Stewart 1992).

In studies conducted at the Equipe de Microbiologie Forestiere, Institut National de la Recherche Agronomique, Centre de Recherches de Nancy, Champenoux, France, nuclear magnetic resonance spectroscopy was used to monitor 15NH4 assimilation and amino acid biosynthesis in *Laccaria bicolor* (strain S238). In mycelium growing rapidly on 15NH4+ (amino-15N), glutamine was the major 14N-labled species. When 15N-labelled mycelium was transferred into medium containing 15NH4+, the resonance for (amino-15N) glutamine decreased with a half-life of about 3 hours, whereas the resonance for (amon-15N) glutamine remained unchanged. Such behavior is consistent with GS being the major route of 15NH4 assimilation. However, the higher accumulation of 15N alanine observed in the presence of the GS inhibitor, methionine sulfoximine, indicated that a part of the glutamate pool was formed by the GDH pathway. When the mycelium was in stationary phase (that is, low extracellular NH4+), the intramolecular 15N labelling of glutamine suggested that the GDH and GS pathways were simultaneously assimilating NH4+. The supply and the growth stage, therefore, influence the expression of the activities of GDH and GS. The current isotopic data identify other fates of absorbed 15N: glutamate decarboxylation gives rise to y-aminobutyrate, transamination between glutamate and pyruvate yields alanine, and arginine accumulates. It is concluded that GS is the main pathway of primary assimilation of NH4+ in *L. bicolor*, but GDH may also contribute significantly to this process (Martin, Cote, and Canet 1994).

Studies conducted at the Institute of Dendrology, Polish Academy of Science, Kornik, Poland, on ammonium assimilation enzymes from several strains of ectendo- and ecto-mycorrhizal fungi were assayed after three weeks culture on a buffered synthetic medium containing ammonium as the sole nitrogen source showed that the activity of NADP-dependent GDH (EC 1.4.1.4) of ectomycorrhizal strains was very low despite excellent mycelial growth. Only ectendomycorrhizal fungus (Mrg X) isolated from roots of pine (*Pinus sylvestris*) showed high GDH activity. Similar results were obtained when the enzyme extracts were subjected to starch gel electrophoresis. Growth of the fungi, except ectendomycorrhizal fungus, was arrested when inhibitors of GS (EC 6.3.1.2), glutamate-ammonia ligase, or glutamate synthetase (GOGAT, EC 1.4.7.1) were included in the culture medium. GS activity was found in all fungi tested. The results suggest that the GS pathway for ammonium assimilation is potentially operative in ectomycorrhizal fungi and imply only a minor role for GDH in ammonium assimilation by the studied ectomycorrhizal symbionts of pine (Rudawska, Kieliszewska-Rokicha, Debaud, *et al.* 1994).

Studies conducted at the University of Queensland, Department of Botany, Brisbane, Australia, on combined gas chromatography-mass spectrometry, used to evaluate the contributions of GDS in nitrogen labelled ammonium assimilation by *Elaphomyces* sp, *Amanita* sp, *Pisolithus* sp and *Gautieria* sp showed that in all the four fungi, glutamine was the major product accumulated following transfer of 14 days old nitrogen limited cultures of fresh medium. The label was rapidly assimilated into fungal tissue, with rates of 733 nmol per g fresh weight/hour in Pisolithus, 972 nmol per g fresh weight/hour in Amanita, 2760 nmol per g fresh weight per hour in Gautieria, and 6756 nmol per g fresh weight per hour in Elaphomyces in the first four hours of incubation. Incorporation of N15 ammonium was sensitive to the inhibitory effects of both MSX (methionine sulphoximine), an inhibitor of GS and albizziin, an inhibitor of GOGAT in Amanita, Gautieria, and Pisolithus, and labelling patterns was consistent with the action of the glulamate synthase cycle in ammonium assimilation and glutamine synthesis was almost totally blocked by MSX. There was no continued incorporation of N15 into glutamate. Elaphomycin displayed high levels of total incorporation of labelled ammonium in mycelium, even in the presence of MSX, although incorporation into glutamine was reduced by 88%. The inhibition of GS by MSX in addition to its partial inhibition by albizziin suggests strongly the action of glutamate synthase cycle in ammonium assimilation. The reduction in label entering glutamate under the influence of albizziin is direct evidence for the inhibition of GOGAT activity. However, MSX treatment had the effect of increasing significantly the quantity of label recovered in both glutamate and alanine. In the absence of GS inhibition, there is clearly competition for ammonium, which, under normal physiological conditions, results in assimilation through the glutamate synthase cycle. However, when GS is blocked by MSX, label is able to cycle through the GDH pathway (Turnbull, Goodall, and Stewart 1996).

Intra specific variations in ammonium-assimilating enzymes

Studies conducted at the Laboratoire de Physiologie Vegetale et Forestiere, Universite de Nancy, Cedex, France, on *Hebeloma cylindrosporum* grown on media containing either glutamate or ammonium as a nitrogen source, revealed that in growth test and in-vitro activity measurement, both GS (EC 6.3.1.2) and NADP-specific GDH (EC 1.4.1.4) were fully functional in wild type mycelia grown on glutamate or ammonium as sole nitrogen source. However, NADP-GDH appeared to be more active than GS in stationary growing mycelia. NADP-GDH was also able to sustain adequate ammonium assimilation in MSX treated mycelia, since they grew as well as mycelia fed with ammonium alone. The NADP-GDH also appeared to be L-glutamate inducible, whereas GS was repressed by ammonium. The NADP-GDH deficient strain of *H. cyclindrosporum,* when transferred from glutamate containing medium to NH4+ containing medium, exhibited a derepressed GS, although this enzyme did not fully substitute for the deficiency of NADP-GDH in ammonium assimilation. The low NADP-GDH activity of the mutant strain exhibited a reduced mobility on a six constant polyacrylamide gel. By contrast, the two enzymes had identical MWs, estimated to be c. 295 kDa on gradient PAGE (polyacrylamide gel electrophoresis) (Chalot, Brun, Debaud *et al.* 1991).

Ammonium assimilation enzymes in vesicular arbuscular mycorrhizal fungi

In studies conducted at the Department of Biology, Fisk University, Nashville, USA, the activity of GS was detected in mycorrhizal spores of *Glomus deserticola* (Jayaraman, Gunasekaran, and Kochhar 1988).

Studies conducted at the Jawaharlal Nehru Vishwa University, Department of Botany, Jodhpur, Rajasthan, India, on *Ziziphus nummularia,* an arid zone fruit plant grown under greenhouse conditions, showed that VAM (vesicular arbuscular mycorrhizal) plants had increased activities of glucamine synthetase, NR, and GDH, besides increasing protein accumulation *in Z. nummularia* (Mathur and Vyas 1995).

Studies conducted at the Department de Microbiologie, Estacion Experimental Station, Granada, Spain, on onion (*Allium cepa*), grown under

well-watered (-0.04 MPa) or drought conditions (-0.17 MPa), inoculated with *Glomus fasciculatum* and supplied with 2 mM nitrogen as nitrate and ammonium in a 1:1 ratio showed that mycorrhizal plants reached a higher specific and total GS activity in shoots and roots than control plants provided with phosphate at -0.04 MPa. The mycorrhizal effect on GS activity under water stress (-0.17 mPa) was evident only in roots being comparable to that found in phosphorus-fertilized plants. The proportion of GS in roots was increased in VAM plants under whatever soil water conditions (Azcon and Tobar 1998).

Studies conducted at the Department of Agriculture, White Agricultural Research Institute, Adelaide, Australia, on crude extracts of roots and shoots of mycorrhizal and non-mycorrhizal *Trifolium subterraneum* and *Allium cepa* grown at different levels of phosphate fertilizer showed that GDH activity was low in all tissues (0.1–1.6 mu.mol NADPH oxidized per minute per gram fresh weight) and there was no consistent effect of mycorrhizal infection or phosphate nutrition on this activity. GS activity (assayed by the transferase method) was in the range 1–40 umol, gamma, glutamyl hydroxamate produced per minute per gram fresh weight. Activity of this enzyme was low in phosphate-deficient plants and was increased both by mycorrhizal infection and by improved phosphate supply. In *T. subterraneum*, the effects of mycorrhizal infection in increasing enzyme activity in roots were similar whether natural soil inoculum with mixture of several VAM fungi or inoculum of *Glomus mosseae* was used. Both increased phosphate supply and mycorrhizal infection increased nodulation and GS activity. It was thus difficult to relate changes in GS activity to the interacting effects of mycorrhizal infection and phosphate nutrition. Onions had low GS activity in uninfected roots, compared with shoots. Improved phosphate supply resulted in increased enzyme activity in both roots and shoots (Smith, St John, Smith, *et al.* 1985).

Effect of nitrogen source on NADP-GDH synthesis

Studies were conducted at the Laboratoire de Microbiology Forestirer, Centre de Recherche, Champenoux, France, to investigate the effects of nitrogen source on the biosynthesis of NADP-GDH with nitrate as the sole nitrogen source in culture medium of *Laccaria bicolor*. NADP-GDH activity, quantity, and biosynthesis rate were measured. Specific activity, polypeptide concentration, and biosynthesis increased when nitrate was the sole source in the culture medium. The enzyme is synthesized de novo. In contrast, NADP-GDH biosynthesis was repressed and optimized and will allow the quantification of NADP-GDH in RNA in mycelium grown on different nitrogen sources. In another experiment, mycelium of *L. bicolor* from NH4+ rich medium was transferred to either $NO3^-$ rich or N-free medium. Under both these conditions, intracellular NH4+ and glutamine content decreased dramatically. The specific activity of NADP-GDH was simultaneously increased 3–4.5-fold. Immunological analysis using anti-GDH antibodies showed that depression of enzyme activity resulted from increased concentration of the GDH polypeptide. In addition, assessment of the steady-state biosynthesis of NADP-GDH by in vivo labelling of proteins demonstrated that the observed changes in enzyme concentration are explained by differences in the relative rate of enzyme biosynthesis. These results suggested that the source of nitrogen regulates synthesis of NADP-GDH and that this regulation is under translational and/or transcriptional control (Lorillou, Tagu, Botton, *et al.* 1993; Lorilloum, Botton, and Nortin 1996).

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Research finding papers

Seasonal population dynamics of vesicular arbuscular mychorrhizal fungi on jatropha planted in wastelands

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Introduction

Jatropha (*Jatropha curcas* L.), a member of the plant family Euphorbiaceae, is the most efficient and economically viable bio-diesel plant. It thrives well in low rainfall regions and in marginal or problem soils. It is a hardy, drought tolerant, and quick growing plant, and is well suited for ecorehabilitation and greening of wastelands (Prasad 2004). More than 80% of the plant species develop a symbiotic relationship with VAM (vesicular arbuscular mycorrhizal) fungi, which helps them to grow well even in phosphorus deficient, moisture stressed, or problem soils (Gerdemann 1968).

Seasons have a direct influence on mycorrhizal root colonization and sporulation (Giovannetti and Nicolson 1983; Smith and Read 1997). The data collected in the present study pertaining to seasonal VAM colonization and sporulation could be helpful in bio-augmentation with resident VAM fungi of jatropha planted in wastelands for their economic management.

Materials and methods

Site selection

The Khandasa farm of N D University of Agriculture and Technology, Kumarganj, Faizabad , which was primarily a wasteland and brought under jatropha cultivation during the last four years, was selected for soil and root sampling. The physicochemical characteristics of the soil are as follows: silty clay loam, sticky and sodic in nature with pH (1:2) 10.0, ECe (ds/m) 12.5, ESP 67.8, hydraulic conductivity (cm/hr) 0.06, organic carbon (%) 0.16, calcium carbonate (%) 4.7, and exchangeable cations (me/100 gm)—sodium 11.8, potassium 0.5, calcium 2.0, and potassium 3.1 (courtesy Soil Science Department).

Sampling

Twenty soil and root samples from one hectare of a jatropha field were randomly collected in the last week of each month starting June 2004 through May 2005. These samples were processed in the laboratory to quantify VAM spores and mycorrhizal colonization of roots.

Quantification of VAM colonization and sporulation

Sporulation soil samples were processed following wet sieving and decanting technique (Gerdemann and Nicolson 1963). To assess the degree of mycorrhizal colonization, thoroughly washed roots of jatropha were stained in Trypan blue after treating them in hot 10% KOH aqueous solution (Phillips and Hayman 1970). The stained roots were cut into 1 cm segments, which were randomly picked up and examined under stereomicroscope for mycorrhizal association. The root colonization was quantified following Nicolson's formula (1955) as follows.

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

Seasonal effect on VAM colonization or sporulation was computed by taking average of values of four months (June–September) for rainy, five months (October–February) for winter, and three months (March–May) for summer seasons.

Identification of VAM fungi

The VAM species were identified on the basis of spore morphology following the manual of Schenck and Perez (1987) and Mukerji (1996).

Table 1 Monthly changes in VA Mycorrhizal root colonization and sporulation on Jatropha planted in wasteland of the university farm

Month	*VAM colonization* (%)	*Sporulation* (spores/100g soil)
June	21.5 ± 8.4	101.3 ±2 8.1
July	45.3 ± 11.3	164.0 ± 38.5
August	52.0 ± 11.7	213.0 ± 54.9
September	50.0 ± 11.3	212.0 ± 52.7
October	30.5 ± 7.7	142.8 ± 36.5
November	24.5 ± 8.4	133.9 ± 34.3
December	15.8 ± 4.8	60.8 ± 11.7
January	12.8 ± 3.7	47.3 ± 12.9
February	16.0 ± 6.6	73.9 ± 17.2
March	22.8 ± 7.0	78.8 ± 19.2
April	56.5 ± 12.1	139.3 ± 45.7
May	20.2 ± 6.8	149.5 ± 53.3

Results and discussions

It is evident from Table 1 that mycorrhizal root colonization and sporulation varied to a great extent. The colonization was 21.5% and spore density 101.3/100 g soil during the month of June, which showed an increasing trend through September with highest colonization 52% and the spore density 213/100 g soil during the month of August. Both colonization and sporulation started to decline from September onwards with highest decrease (12.8% colonization and 47.3 spores/100 g soil, respectively) during January. These started to increase again from February with highest colonization (56.5%) during April and sporulation (149.5 spores/100g soil) during May.

The root colonization (Figure 1) and sporulation (Figure 2) fluctuated to a great extent with seasons also. These were lowest (colonization 19.9% and sporulation 91.7 spores/100 g) during the winter and highest (colonization 42.2% and sporulation 172.6 spores/100 g soil) during the rainy seasons.

Proliferation of root system and luxuriant growth of plant occurring during the rainy season could be attributed to high relative humidity (90%–95%) and leaching of salts from soil. Increased level of mycorrhizal colonization in other plant species and sporulation during this season has been reported (Mason, Musoko, and Last 1992; Raghupathy and Mahadevan 1993; Bhaskaran and Selvaraj 1997; Allen E B, Rincon, Allen M F, *et al.* 1998). However, an abrupt fall in root colonization and sporulation during the winter season could be ascribed to senescence in plants which resulted in reduced root exudation and

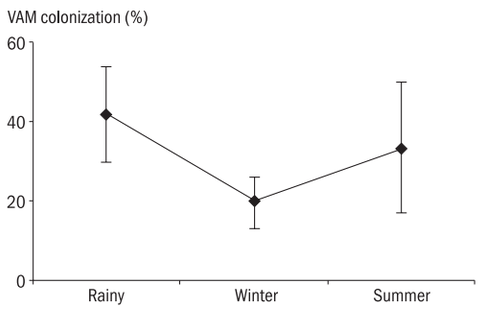

Figure 1 Effect of seasons on VAM colonization of jatropha roots (root colonization)

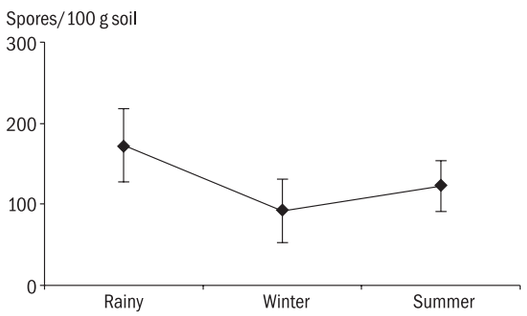

Figure 2 Effect of seasons on VAM sporulation around jatropha (sporulation)

to the fruiting stage during which most of the photosynthate was allocated to the aerial parts where it was urgently needed for the development of fruits rather than to the roots. Thus, reduced root exudation and limited carbohydrate allocation to the roots rendered VAM fungi starved of carbon source, which might have adversely affected the root colonization and sporulation. Giovannetti (1985), Puppi, Tabachini, Riess, *et al.* (1986), and Siquenza, Spejel, and Allen (1996) have also arrived at a similar conclusion. Immediately after the onset of summer, the dormancy was broken and plants started to bloom signalling the greatest metabolic activities resulting in profuse root exudation, which might have favoured mycorrhizal root colonization and sporulation (Koske 1981; Warner and Mosse 1980).

Out of various VAM species identified (*Glomus mosseae*, *G. fasciculatum*, *Gigaspora margarita*, *G. gigantea*, and *Acaulospora laevis*), *G. mosseae* was found to be the most predominant species as it occurred in 71.3% of the samples analysed. This may be due to adaptation of this fungus to alkaline soils (Mosse 1973).

If bio-augmentation with VAM fungi of jatropha planted in wastelands is to be carried out, the rainy season should be preferred (as root colonization and sporulation were highest) as compared to winter or summer seasons.

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Response of rice crop inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria to different soil nitrogen concentrations

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Introduction

AMF (arbuscular mycorrhizal fungi) is widely distributed in agro-ecosystems (Smith and Read 1997), forming symbiotic associations with the roots of plants. They play an important role in plant mineral nutrition and plant health (Barea, Azcòn, and Azcòn-Aquilar 2002; Ferrol, Barea, and Azcòn-Aquilar 2002; Giovannetti, Sbrana, and Avio 2002). These fungi have a wide range of application in sustainable low input agricultural systems (Schreiner and Bethlenfalvay 1995). The use of AMF may contribute to reducing chemical fertilizer inputs and sustaining plant productivity in agriculture (Mc Gonigle 1988). In natural soils in the presence of indigenous fungi, introduced isolates differ in their ability to stimulate plant growth (Medina, Sylvia, and Kretschmer 1988).

The contribution of PGPR (plant growth promoting rhizobacteria) in phytostimulation, phytoremediation, and biofertilization is well documented (Goldstein 1986; Kloepper, Lifshitz, and Schroth 1988; Glick 1995; Barea 2000; Tank and Saraf 2003). *Azotobacter is* regarded as a broad-spectrum inoculant as it could be used for inoculating wide variety of crops such as wheat, rice, sorghum, barley, potato, sugarbeet, cotton, maize, etc. (Rai and Gaur 1988; Martinez-Toledo, de la Rubia, Moreno *et al.* 1988; Tiwari, Lehri, and Pathak 1989). PGPR interact synergistically with VAM (vesicular arbuscular mycorrhizal) fungi and promote plant growth (Bagyaraj and Menje 1978).

The present study, therefore, was conducted under net house conditions using unsterilized soils, to test the effectiveness of the introduced AMF and plant growth promoting rhizobacteria in the presence of their indigenous counterparts on rice grown at different soil nitrogen concentrations.

Materials and methods

Soil

Test soil was collected from rice fields located near Gauhati University, Guwahati, Assam, India. It contained 0.038% phosphorus (total), 0.106% nitrogen (total), and 1.3 PPM (parts per million) potassium with pH_w 5.77, and moisture content of 38%. Earthen pots of 20 cm diameter with a drainage hole were filled with approximately 5 kg of soil.

Experimental design and treatments

The pot experiment was conducted in the net house in the botanical garden, Department of Botany, Gauhati University, Guwahati. The CRB (completely randomized block) design was used for the experiment. There were 16 soil treatments with control, *Glomus* only, *Azotobacter chroococcum* only, and *Glomus* + *Azotobacter chroococcum* only, at four different levels of nitrogenous fertilizer; that is, zero kg/ha, 44 kg/ha, 88 kg/ha, and 132 kg/ha. These 16 soil treatments were replicated three times. Nitrogen was applied in three splitted doses. The first dose, consisting of 1/3 the normal dose, was applied before transplantation; the second 1/3 at the time of tillering; and the last 1/3 at the panicle initiation phase.

Collection of seeds and raising seedlings

Rice (*Oryza sativa* L. var. Lachit) seeds were collected from the Department of Agriculture, Government of Assam. Seeds were surface sterilized with 1%–2% sodium hypochlorite for 1 minute followed by 70% alcohol for 30 seconds. Thereafter, they were washed several times with double distilled sterilized water. They were soaked in sterile water and then allowed to germinate on cotton cloth. They were sown on sterilized sand on trays after germination. When the seedlings were three weeks old, three seedlings were transplanted into pots with different treatments.

Bioinoculants used

The microbial inocula used were *Glomus sp.* and *Azotobacter chroococcum*. *Glomus sp.* was isolated from different rice rhizospheres. The mycorrhizal inoculum consisting of spores, soil, and infected root fragments were obtained from the pot cultures of *Chloris gayana* Kunth. as the host plant. Each pot received an inoculum of 10 g at 2 cm below the soil surface near the root system. The non-mycorrhizal pots received the same quantity of autoclaved inoculum.

Azotobacter chroococcum, was isolated from the rice rhizosphere using Ashby's medium and maintained on agar medium as *Azotobacter chroococcum* was enriched on nutrient media. After 24 hours, the cells were collected by centrifugation at 2000 rpm for 20 minutes and suspended in ¼ strength Ringer's solution so as to get 5×10^5 cells ml^{-1} by using haemocytometer. Each *Azotobacter chroococcum* treated pot obtained 3 ml of this cell suspension.

Measurement

Plant height and tiller number were recorded thrice at an interval of 30 days after 1 month of transplantation. Ear number and 1000-grain weight were recorded after harvesting. Shoot and root dry weights were recorded after drying the plants in an oven at 70°C for 48 hours and then cooling them in a dessicator. Spore count (50 g/ads [amended soil]) was estimated by the wet sieving and decanting method (Gerdemann and Nicolson 1963) and the percentage of root length colonized by AMF was estimated by examining stained samples (Koske and Gremma 1989) microscopically (Brundrett, Piche, and Peterson 1984).

Determination of plant nutrient concentration

The shoot phosphorus (P%) and nitrogen content (N%) of the plants were determined by using the ascorbic acid procedure as described in the *Laboratory methods of soil and plant analysis: a working manual* (Okalebo 1993) and Indophenol Blue Method (Allen 1974), respectively, after an acid digestion treatment.

Statistical analysis

Results were subjected to two-way analysis of variance and the significance was determined according to Duncan's Multiple Range Test (Gomez K A and Gomez A A 1984).

Results

Rice plants varied in their response to inoculation with *Glomus*, *Azotobacter chroococcum*, and their combinations. Generally, inoculated rice plants had greater growth compared to uninoculated controls (Table 1). Rice plants inoculated with *Glomus* sp. attained maximum height when nitrogen was added at 88 kg/ha. The *Azotobacter chroococcum* inoculated plants, however, required less nitrogen, that is, nitrogen at 44 kg/ha to attain height at par with *Glomus* sp. inoculated plants. The dually inoculated plants attained maximum height when soil was fertilized with nitrogen at 88 kg/ha (Table 1).

Dually inoculated plants irrespective of rate of nitrogen application produced maximum shoot biomass. Inoculation of rice plants with *Azotobacter chroococcum* either alone or in combination with *Glomus* sp. produced maximum shoot biomass. Increasing soil nitrogen levels had a negative impact on shoot biomass in both inoculated and uninoculated plants. Efficiency of mycorrhizal fungi was significantly improved when they were used along with *Azotobacter chroococcum.*

The positive effect of microbial inoculants on tiller number, ear number, and grain yield were observed at all the levels of nitrogen-fertilization. The maximum number of tillers and ears was observed at a low level of nitrogen addition (44 kg/ha) in all microbial treatments (Table 1). More grain yield was also recorded from plants growing on low levels of nitrogen addition. Rhizobacterization of plants with *Azotobacter chroococcum* resulted in a significant increase in tiller number irrespective of whether they were mycorrhizal or non-mycorrhizal. Higher levels of soil nitrogen were inhibitory for growth and yield of rice, irrespective of the nature of microbial inoculants used (Table 1). No significant difference was observed between *Glomus* sp. and *Azotobacter chroococcum* for their ability to induce tiller production at the same level of soil nitrogen. However, *Azotobacter chroococcum* could produce more ears than *Glomus* sp. at the highest level of nitrogen concentration.

There is significant increase in shoot phosphorus content in the inoculated plants compared to uninoculated control at all the levels of nitrogen fertilization (Table 1). Plants inoculated dually with *Glomus* sp. and *Azotobacter chroococcum* possessed higher shoot phosphorus content than the singly inoculated ones. The highest shoot nitrogen content was also recorded from dual inoculated plants growing on highest soil nitrogen concentrations.

The endogonaceous spore population was more in inoculated plants than uninoculated control plants, irrespective of the soil nitrogen concentrations (Figure 1). Rice plants inoculated with *Glomus sp.* harboured more spore numbers than *Azotobacter chroococcum* inoculated ones. Spore numbers in the rhizosphere of dually inoculated rice plants were significantly higher than those of the singly inoculated ones. The maximum spore number was recorded from the rhizosphere of dual inoculated plants growing on 44 kg/ha of nitrogen application and the minimum spore number was recorded from uninoculated unfertilized plants.

The root colonization of inoculated rice plants increased significantly from the uninoculated control ones (Figure 2). *Glomus* sp. only inoculated plants had more roots infected as compared to *Azotobacter chroococcum* only inoculated plants growing on all the levels of soil nitrogen concentrations. More roots were colonized when plants were inoculated with both the inoculants than with only *Glomus*. The uninoculated

Table 1 Growth of rice plants as influenced by Glomus sp. and Azotobacter chroococcum at different concentrations of N-fertilizer

Bicrobial inoculums	*N-level* (kg/ha)	*Height* (cm)	*Biomass* (g)	*Tiller* no.	*Ear* no.	*1000 grain* weight (g)	*Shoot N* (%)	*Shoot P* (%)
	0	74.00	10.00	4.00	5.00	17.64	2.08	0.073
	(e)	(f)	(i)	(h, i)		(e, f)	(j)	(j)
	44	82.00	15.45	5.00	10.00	19.07	2.70	0.081
		(c, d, e)	(e)	(g, h, i)	(d ,e)	(c, d, e)	(g, h)	(g,h)
Control	88	87.33	11.00	4.66	4.00	18.91	3.19	0.090
		(b, c)	(f)	(h, I)	(h, I)	(c, d, e)	(c, d, e)	(c, d)
	132	76.00	8.50	3.00	3.00	13.25	3.13	0.076
		(d, e)	(g)	(i)	(j)	(g)	(d, e)	(i)
	0	75.33	14.20	6.00	9.00	19.00	2.19	0.084
		(e)	(e)	(f, g, h)	(e, f)	(c, d, e)	(i, j)	(e, f)
	44	87.66	25.34	12.00	15.00	20.89	2.93	0.093
		(a, b, c)	(b)	(b, c)	(b)	(b, c, d)	(f)	(b, c)
Glomus sp.	88	96.33	21.64	9.00	10.00	19.80	3.28	0.089
		(a)	(c)	(d,e)	(d,e)	(b,c,d,e)	(b,c,d)	(d)
	132	87.00	11.35	5.00	5.00	15.66	3.36	0.07
		(b, c)	(f)	(g, h, i)	(h, i)	(f)	(a, b, c)	(h)
	0	77.00	15.00	6.00	10.00	20.68	2.25	0.079
		d, e)	(e)	(f, g, h)	(d, e)	(b, c, d)	(i)	(h)
	44	91.66	32.67	12.00	16.00	21.19	3.08	0.085
		(a, b)	(a)	(b, c)	(a, b)	(a, b, c)	(e, f)	(e, f)
Azotobacter *chroococcum*	88	87.66	20.00	8.00	11.00	20.74	3.35	0.094
		(a, b, c)	(c, d)	(d, e, f)	(c, d)	(b, c, d)	(a, b, c)	(b)
	132	78.00	16.00	7.00	7.00	17.20	3.40	0.082
		(d, e)	(e)	(e, f, g)	(g)	(e, f)	(a, b)	(f, g)
	0	78.00	18.50	7.00	12.00	22.38	2.62	0.092
		(d, e)	(d)	(e, f, g)	(c)	(a, b)	(h)	(b, c, d)
	44	84.00	34.15	18.00	18.00	23.82	3.28	0.10
		(b, c, d)	(a)	(a)	(a)	(a)	(b, c, d)	(a)
Glomus sp. + Azotobacter *chroococcum*	88	90.33	27.00	14.00	16.00	22.50	3.44	0.103
		(a, b, c)	(b)	(b)	(a, b)	(a, b)	(a, b)	(a)
	132	76.33	19.50	10.00	8.00	18.40	3.52	0.085
		(d, e)	(d)	(c, d)	(f, g)	(d, e)	(a)	(e, f)

Means with the same letter in a column are not significantly different according to DMRT, P < 0.05

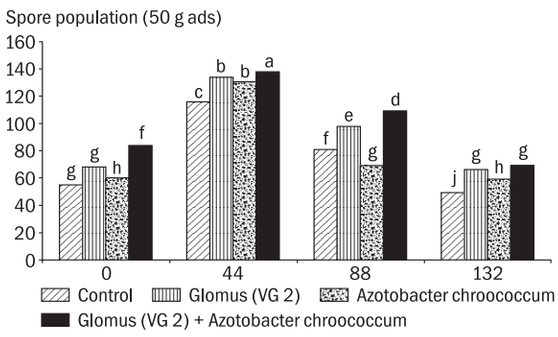

Figure 1 Effect of Glomus sp and Azotobacter chroococcum on endomycorrhzal spore population. Bars with same letter(s) do not differ significantly according to Duncan's Multiple Range Test at P < 0.05.

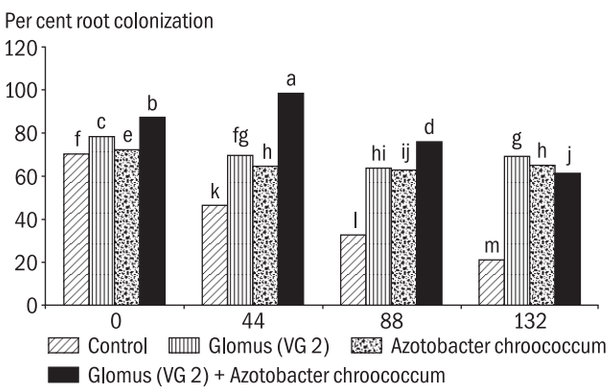

Figure 2 Effect of Glomus sp and Azotobacter chroococcum on percentage root infection on rice plants. Bars with same letter(s) do not differ significantly according to Duncan's Multiple Range Test at P < 0.05.

control plants growing on 132 kg ha^{-1} of nitrogen amended soil had lowest root per cent infection. The percentage of total root infected was more in plants with no nitrogen application, irrespective of microbial treatments. Both mycorrhizal spore population and root infection by mycorrhizal fungi were significantly influenced by microbial treatments as well as nitrogen concentrations in soil.

The analysis of variance showed a significant ($P < 0.05$) interaction effect between nitrogen addition and microbial inoculation, indicating that the differences in plant growth due to microbial infection were not same at different rates of nitrogen and, similarly, that nitrogen responses differed between inoculated and uninoculated plants.

Discussion

In this experiment, urea was taken as the source of nitrogenous fertilizer because it is a satisfactory source of nitrogen for cereals (Lloyd, Webb, Archer, *et al.* 1997). It has been observed that sets inoculated with *Glomus* sp. and *Azotobacter chroococcum*, either alone or in combination with different levels of soil nitrogen application, positively affected crop growth over the treatments that received nitrogen only. As observed earlier (Azcon, Ruiz–Rozano and Rodriguez 2001), increasing nitrogen application had a negative effect on plant height, irrespective of whether the plants were inoculated or not. The present result indicates that inoculation of rice plants with *Glomus* sp. and, the dual inoculation of *Glomus* sp. and *Azotobacter chroococcum* enhanced the plant growth when urea was incorporated up to the recommended dose—that is, 88 kg/ha.

Rice plants inoculated with *Glomus* sp., either alone or in combination with *Azotobacter chroococcum,* grew taller than the uninoculated control plants. This is in agreement with earlier works on cotton, maize, and soybean grown on unsterilized soil (Mohan, Bagyaraj, and Manjunath 1984). Rice plants for tiller production responded more to dual inoculation of AMF and only PGPRs than to either only mycorrhizal fungi or only PGPR. The present results, together with previous reports (Zambre, Konde, and Sonar 1984), confirm that association of crop yields with AMF and PGPRs enhanced the number of tillers per pot. Dhillion, Kler, and Chahal (1980); Rai and Gaur (1982); and Zambre, Konde, and Sonar (1984) also reported that a greater number of tillers was produced when the crop was inoculated with PGPR. The shoot dry weight of rice plant significantly differed from each other at every level of nitrogen fertilization. Rhizobacterization is known to increase the shoot dry weight in nitrogen-fertilized soils (Raut and Ingle 1980; Subba Rao, Tilak, Lakshmikumari, *et al.* 1980; Zambre, Konde, and Sonar 1984; Boddey, Baldani V D, Baldani J, *et al.* 1986). Non-inoculated control plants had significantly lower dry weight than plants colonized by AMF, indicating that rice crop was positively responsive to VAM inoculation. Azcon, Ruiz–Rozano, and Rodriguez (2001) made similar observations. Inoculated crops showed retarded growth when nitrogen was added at 132 kg nitrogen ha^{-1}. This may be because the high dose of inorganic nitrogen might inhibit development of free living and diazotrophic bacteria. Rice plants inoculated with mycorrhiza and/or rhizobacteria insignificantly increased the 1000-grain weight over uninoculated control. Reyndars and Vlassac (1982); Kundu and Gaur (1980); and Zambre, Konde, and Sonar (1984) have also reported that inoculation of wheat with *Azospirillum* and *Azotobacter* increased grain yield. Such growth responses are variable and depend upon the initial fertility status of soil and the type of crop planted (Subba Rao, Tilak, Lakshmikumari, *et al.* 1980). The increase in growth and yield of *Azotobacter chroococcum* inoculated plants is not necessarily due to the nitrogen fixation by the added rhizobacteria (Lethbridge and Davidson 1983), but might also be due to the growth hormones secreted by the rhizobacteria (Tien, Gaskins, and Hubbell 1979) and tropical growth conditions (Wani, Chandrapalaiah, and Dart 1982).

In unsterilized soil, inoculated plants showed higher spore population and higher percentage of root infection compared to the uninoculated control plants. This indicates that inoculation with mycorrhizal fungi stimulated rice growth beyond indigenous AMF. This might be because of low indigenous AMF, which allowed effectiveness, introduced AMF to be adequately tested (Mohammad, Mitra, and Khan 2004).

It was hypothesized that AMF would be effective only for the acquisition of slowly diffusing nutrients by plants (Harley and Smith 1983). However, the present work demonstrates the effectiveness of mycorrhizal roots to promote nitrogen uptake, even when nitrogen, in the form of urea, is present in the soil in non-limiting amounts. This is in agreement with the recent findings of Azcon, Ruiz–Rozano, and Rodriguez (2001) that mycorrhizal roots promote nitrogen uptake when present in non-limiting amounts.

The addition of nitrogen at 44 kg ha^{-1} improved the growth parameters in inoculated plants. The statistically significant interaction between nitrogen addition and microbial treatments suggests that the differences in growth parameters owing to different microbial treatments were due to different rates of nitrogen application and the nitrogen response differed between inoculated and non-inoculated plants. The results of the present work strongly suggest that application of bioinoculants like AMF plus PGPR would enable farmers for optimizing rice production with minimum input of inorganic fertilizer.

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Cumulative effect of arbuscular mycorrhizal fungi, vermicompost, and Trichoderma harzianum on bunch, finger characters, and yield of banana cv. Rajapuri (Musa AAB)

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Introduction

Owing to continuous vegetative growth, and flowering and fruiting habits, the nutrient requirement of banana is high and this is supplemented with chemical fertilizers. The chemical fertilizers are not only costly, but pollute soil and ground water, which is a main constraint in their liberal use. The contribution of symbionts like AM (arbuscular mycorrhizal) fungi in increasing the growth and yield, and in reducing the fertilizer requirement have been brought out by few workers in fruit crops (Adivappar 2001). The literature also reveals that AM fungal efficacy can be increased with the aid of vermiculture technology (Kale, Bano, Sreenivasa, *et al*. 1987). Therefore, the present investigation was undertaken to find out the interaction effect of AM fungi, vermiculture, and *Trichoderma harzianum* on bunch, finger, and yield characters of banana cv. Rajapuri.

Material and methods

A field experiment was conducted at the Department of Pomology, Kittur Rani Channamma College of Horticulture, Arabhavi, during 2000–02. It was laid out in split plot design with mycorrhiza (*Glomus fasciculatum*) and without mycorrhiza as main treatments, while *in-situ* vermiculture (200 000 t/ha), vermicompost (1 kg/plant) along with 50% and 75% RDF (recommended doses of fertilizers), and RDF + *Trichoderma harzianum* as five sub-treatments. The treatments were replicated thrice. The banana plants were inoculated by placing 50 g AM fungal culture (*G. fasciculatum*) with a minimum of 19 infective spores per gram culture in the pit before the planting of suckers. The earthworms (*Eudrillus enginae*) were released after the planting of suckers at the rate of 50 per plant. The required doses of fertilizer were applied in three splits at one, three, and five months, after planting as per the

treatments. The RDF of 180:108:225 g of NPK (nitrogen phosphorus potassium) per plant was adopted in the experiment (Anonymous 2002).

Observations on bunch characters like bunch weight, bunch length, bunch width, number of fingers per hand, number of hands per bunch, total number of fingers per bunch; finger characters like finger length, girth, weight, pulp weight, peel weight, and pulp-to-peel ratio; and yield characters were recorded. The data recorded on various characters were subjected to Fisher's methods of analysis of variance.

Results and discussion

The *Glomus fasciculatum* inoculated plants recorded significantly maximum bunch weight, number of hands per bunch, total number of fingers per bunch, finger length, finger weight, pulp weight, and yield per hectare when compared to uninoculated plants (Tables 1, 2, and 3). Among the sub-treatments, plants applied with vermicompost + 75% RDF recorded maximum bunch weight, total number of fingers per bunch, finger weight, and yield, and plants applied with *in-situ* vermiculture recorded the maximum number of hands per bunch, finger girth, pulp weight, and pulp-to-peel ratio. In the interaction effects, AM fungal inoculated plants along with vermicompost + 75% RDF recorded maximum bunch weight, number of fingers per hand, and yield, while AM fungal inoculated plants along with *in-situ* vermiculture recorded the maximum number of hands per bunch, total number of fingers per bunch, finger girth, finger weight, pulp weight, and pulp-to-peel ratio.

An increased growth rate of banana with inoculum of mycorrhiza has been reported by many workers (Alonso, Gonzalez, Exposito, *et al*. 1995; Parvathareddy, Nagesh, Rao, *et al*. 1997). Further, growth of mycorrhizal plants is usually high when the potential for active photosynthesis is high. The high rate of photosynthesis by mycorrhizal plants may be evoked by a number of changes, such as an increase in plant hormones (Miller 1971), stomatal opening, enhanced ion transport, and regulation of chlorophyll level (Johnson 1984). Further, increased uptake of nitrogen, phosphorous, potassium, copper, manganese, iron, and zinc (Bagyaraj and Manjunath 1980) and increased tolerance to biotic stresses (Abdulla 2002) might have contributed to the production of more leaves and greater leaf area, and thereby, higher photosynthetic capacity during reproductive phase and translocation of carbohydrates from other plant parts to reproductive parts which might have been resulted in increased yield and yield attributes.

The increase in yield and yield attributing parameters in the treatments applied with vermicompost + 75% RDF could be attributed to the worm cast, which is a rich source of micronutrients, vital plant promoting substances, nitrogen-fixers, and other beneficial microorganisms, which are known to increase the vigour and yield of the plant (Venkatesh, Patil, Athani, *et al*. 1998), thereby replacing 25% RDF.

The better performance of combination of mycorrhiza, vermiculture and 75% RDF may be attributed to the individual and interactive effects. According to Kale, Bano , Sreenivasa, *et al*. (1987), the mycorrhizal efficiency increases with vermicompost.

The present investigation, therefore, clearly indicates that the use of *Glomus fasciculatum* with vermicompost + 75% RDF will increase the banana yield with saving in RDF by 25%.

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Table 1 Influence of AM, vermiculture, and *Trichoderma harzianum* on bunch characteristics of banana

Treatment	*Bunch weight* (kg)			*Bunch length* (cm)			*Bunch width* (cm)			*Number of fingers per hand*			*Number of hands per bunch*			*Total number of fingers per bunch* (kg)		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
S_1	8.75	7.50	8.13	40.12	38.58	39.35	24.92	24.58	24.75	12.14	11.81	11.98	8.23	7.63	7.93	96.22	86.45	91.34
S_2	10.50	8.51	9.51	38.67	37.67	38.17	24.44	25.31	24.88	12.35	11.52	11.94	7.60	7.53	7.56	94.10	91.11	92.61
S_3	8.12	7.25	7.69	40.05	39.47	39.76	25.04	25.10	25.07	11.71	10.90	11.30	7.40	7.31	7.35	85.63	83.17	84.40
S_4	9.00	8.75	8.88	38.93	40.97	39.95	24.55	25.22	24.89	12.05	12.31	12.18	7.64	7.59	7.62	94.45	89.85	92.15
S_5	9.50	8.38	8.94	39.56	38.22	38.89	24.29	23.72	24.00	12.17	12.11	12.14	7.71	7.56	7.64	94.08	89.42	91.75
Mean	9.18	8.08		39.47	39.98		24.65	24.79		12.08	11.73		7.71	7.52		92.89	88.00	
For comparing	S.Em	C.D.		S.Em	C.D.		S.Em	C.D.		S.Em	C.D.					S.Em	C.D.	
the means of	±	at 5%		±	at 5%		±	at 5%		±	at 5%					±	at 5%	
Main (M)	0.103	0.629		0.485	NS		0.042	NS		0.097	NS		0.016	0.099		0.060	0.367	
Sub (S)	0.171	0.511		0.473	NS		0.347	NS		0.110	0.328		0.041	0.123		0.826	2.476	
S at same M	0.241	0.723		0.669	NS		0.491	NS		0.155	0.464		0.058	0.174		1.168	3.501	
M at same S	0.239	0.717		0.770	NS		0.441	NS		0.169	0.507		0.054	0.163		1.047	3.137	

M_1 – With AM; S_1 – *In-situ* vermiculture; S_4 – RDF; M_2 – Without AM; S_2 – Vermicompost + 75% RDF; S_5 – RDF + *Trichoderma harzianum;* S_3 – Vermicompost + 50% RDF; NS - Non-significant

Table 2 Influence of AM, vermiculture, and *Trichoderma harzianum* on finger characteristics of banana

Treatment	*Finger length* (cm)			*Finger girth* (cm)			*Finger weight* (g)			*Pulp weight* (g)			*Peel weight* (g)			*Pulp to peel ratio*		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
S_1	14.92	14.00	14.46	3.52	3.05	3.28	90.00	79.72	84.86	64.83	51.18	58.01	30.60	28.21	29.41	2.12	1.82	1.97
S_2	15.48	14.18	14.83	3.14	2.89	3.02	86.53	83.46	84.99	57.55	55.43	56.49	29.88	28.93	29.41	1.93	1.92	1.92
S_3	14.96	13.97	14.46	2.92	2.81	2.86	78.90	76.02	77.46	52.19	50.64	51.42	29.28	28.46	28.87	1.79	1.79	1.79
S_4	15.32	15.15	15.24	3.15	2.98	3.07	71.74	66.25	68.99	46.30	43.75	45.03	29.29	28.65	28.97	1.59	1.53	1.56
S_5	14.92	14.17	14.54	3.10	3.07	3.09	70.32	70.27	70.29	44.11	44.17	44.14	29.25	29.14	29.19	1.51	1.52	1.51
Mean	15.12	14.29		3.17	2.96		79.50	75.14		53.00	49.04		29.66	28.68		1.79	1.71	
For comparing	S.Em±	C.D.		S.Em	C.D.		S.Em±	C.D.		S.Em	C.D.		S.Em	C.D.		S.Em	C.D.	
the means of	±	at 5%		±	at 5%		±	at 5%		±	at 5%		±	at 5%		±	at 5%	
Main (M)	0.125	0.758		0.038	NS		0.558	3.393		0.404	2.461		0.272	NS		0.017	NS	
Sub (S)	0.260	NS		0.037	0.111		0.737	2.209		0.416	1.247		0.377	NS		0.026	0.078	
S at same M	0.367	NS		0.052	0.157		1.043	3.124		0.589	1.764		0.532	NS		0.037	0.110	
M at same S	0.351	NS		0.061	0.182		1.086	3.256		0.664	1.989		0.548	NS		0.037	0.111	

M_1 – With AM; S_1 – *In-situ* vermiculture; S_4 – RDF; M_2 – Without AM; S_2 – Vermicompost + 75% RDF; S_5 – RDF + *Trichoderma harzianum;* S_3 – Vermicompost + 50% RDF; NS– Non-significant

Table 3 Influence of AM, vermiculture, and *Trichoderma harzianum* on yield of banana

	Yield					
	kg/plot			t/ha		
Treatment	M_1	M_2	Mean	M_1	M_2	Mean
S_1	87.50	75.00	81.25	27.01	23.15	25.08
S_2	105.00	85.10	95.05	32.41	26.27	29.34
S_3	81.23	72.50	76.87	25.08	22.37	23.72
S_4	90.00	87.50	88.75	27.78	27.01	27.39
S_5	95.00	83.77	89.38	29.32	25.97	27.65
Mean	91.75	80.77	28.32	24.95		
For comparing	S.Em	C.D.		S.Em±	C.D.	
the means of	±	at 5%		±	at 5%	
Main (M)	1.034	6.293		0.305	1.856	
Sub (S)	1.707	5.115		0.529	1.586	
S at same M	2.414	7.234		0.749	2.243	
M at same S	2.394	7.174		0.736	2.205	

M_1 – With AM; S_1 – *In-situ* vermiculture; M_2 – Without AM; S_2 – Vermicompost + 75% RDF; S_3 – Vermicompost + 50% RDF
S_4 – RDF; S_5 – RDF + *Trichoderma harzianum*; RDF – recommended dose of fertilizer

New approaches

Integration of double pot and double compartment techniques for nutritional studies in mycorrhizal fungi

The double compartment technique is used in studies on nutrient uptake by mycorrhizae, whereas the double pot technique is used to assess the nutritional stress of plants grown in different soils. A combination of the double pot and the double compartment technique was used by Cardoso I M, Boddington C L, Janssen B H, Oenema O, and Kuyper T W (2004) as a tool to understand the processes involving mycorrhiza and plant nutrition. Maize (*Zen mays*) and three species of *Glomus* were used to study phosphorus uptake with and without mycorrhiza from the A and B horizons of an Oxisol. The plants were supplied from the lower pot with a nutrient solution without phosphorus. The upper pot had a double compartment with either a fine or coarse mesh screen to control the volume of soil explored by the roots, and thereby, limit the amount of soil phosphorus accessible to plants from the test soil. There were significant effects of time for plants grown in both soil horizons, and of mycorrhizae for plants grown in the A soil horizon. No effect of mesh size was observed. There were significant effects of horizon and mycorrhiza, but not of mesh size, on the dry weight and phosphorus content of shoots and roots. The phosphorus concentration for shoots and roots was similar in the A and B soil horizons. The results showed that the double pot–double compartment system was suitable for the experimental objectives. No differences in plant growth were observed when root growth was not limited, versus root growth limited to the inner compartment, because the non-mycorrhizal plants did not take up phosphorus. Consequently, the responsiveness of the maize cultivar was wholly dependent on mycorrhiza for phosphorus uptake under these experimental conditions.

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Centre for Mycorrhizal Culture Collection

Development of green cover at solid waste dumping site of a soda lime industry by mycorrhizal technology

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Introduction

Soil microorganisms are important in the recovery of disturbed and potentially toxic environments because they produce plant growth stimulating substances such as hormones and vitamins, immobilize heavy metals in the soil, bind soil particles into stable aggregates which improve soil structure, reduce erosion potential, and can contribute to nutrient availability to plants (Gadd 1993; Shetty, Hetrick, Figge *et al.* 1994). The AMF (arbuscular mycorrhizal fungi) are an important component of the soil microbial biomass. The symbiosis is mutualistic based on bi-directional nutrient transfer between the symbionts. The plant benefits particularly through enhanced phosphorus, and water and mineral nutrient uptake (Smith and Read 1981), which often results in better growth. The AMF can protect plants against the toxic effects of excessive concentrations of heavy metals (Heggo, Angle, and Chaney *et al.* 1990; Marschner 1995).

Wastelands are the drastically disturbed lands where native vegetation and animal communities have been removed and the topsoil has been lost, altered, or buried. Examples of wastelands are surface mined lands for minerals (coal, pauline, phosphate, bauxite, gravel, sand, etc.), mining wastes, organic/inorganic solid waste dumping sites of various industries, top soil removed for use elsewhere creating 'burrow pits', saline soils, degraded material, and wind and water eroded sites. Natural rehabilitation of such lands is a gradual process. AM (arbuscular mycorrhiza) is a potential tool for reclamation of wastelands owing to its beneficial effects. Such association in terms of their growth rate, tolerance against biotic and abiotic stresses, and seedling survival benefit the plant species. These microorganisms provide nutrition to the plant by sequestering the nutrients from the soil and translocating them to the plant, and, in return, get carbon from the plants. This makes the utilization of the nutrients highly efficient and reduces the dependence on external chemical inputs. Therefore, the mycorrhizal technology offers a biological means of assuring plant production at a low cost without chemical fertilizers.

Plants can be used in the remediation of contaminated soils. In fact, plants have mechanisms for accumulation, tolerance, or alleviation of high levels of heavy metals in contaminated soil (Khan, Kuek, Chaudhry *et al.* 2000). Under field conditions, different plant species live together and hyphae of AMF interconnect the root systems of adjacent plants, changing the level of AM colonization. AM hyphae can mediate nutrient transfer between plants (Bethlenfalvay, Schreiner, Mihara *et al*. 1996; Ocampo 1986).

The study described in this paper focused on the contribution of AM symbiosis to reclaim the solid waste generated by soda ash industry and develop a green cover by growing different plant species.

Materials and methods

The present study was carried out in the premises of Tata Chemical Ltd, Mithapur, which is located in the Dwarka sub-division of Gujarat state on the west coast of India. Tata Chemicals produces 2400 TPD (tonnes per day) soda ash, 1500 TPD of vacuum-evaporated salt, and 33 other products, and generate a huge amount of solid waste. These solid wastes are highly alkaline in nature, with a large quantity of salts, and very low in nitrogen, phosphorus, and potassium (Table 1).

Plant species viz. desi babool, ram babool, casurina, and paras papal were selected for plantation at a solid waste dumping site on top of the ridges (1.0 m base, 0.75 m height, 0.75 m top). Plants were planted in 0.45 m, 0.45 m, 0.45 m size pits, using 3 kg aerobic compost along with 200 propagules/ plant. The drip system was installed for irrigation and 2 liters of water was provided daily to each plant. Soil samples were taken before plantation and after one year of plantation, and were analysed for pH, EC, OC (organic carbon), nitrogen, phosphorus, and

Table 1 Changes in physico-chemical characteristics of solid waste before and after plantation.

Parameters	*Initial*	*After one year*			
		Desibabool	*Rambabool*	*Casurina*	*Paras pipal*
pH	11.45	7.35	7.26	7.15	7.35
EC (ms/cm)	74.10	53.25	62.40	23.65	65.45
OC (%)	1.02	2.56	2.62	2.75	2.61
N (%)	0.01	0.75	0.21	0.24	0.37
P (mg/kg)	7.64	39.62	29.12	38.65	37.15
K (mg/kg)	68.00	318.65	161.54	175.65	165.45

N – nitrogen; P – phosphorus; K – potassium

potassium by following standard methods. The height and girth of plants at 37 cm height was observed after one year.

Results and discussion

The Mycorrhiza biofertilizer is necessary for augmenting the nutritional capabilities of the plant roots. Bio-inputs at various stages of greening operation of wasteland overburdens can provide additional benefits. For example, decomposition of green vegetation serving as green manure can be quickened by specific bio-inputs. Mycorrhiza has benefited both the plants and the soil. Plant benefits include augmentation of the supply of phosphorus and trace elements (iron, boron, zinc, copper, etc.), protection of plant roots from root diseases, high soil temperatures, and high salt concentrations, amongst others. Fungal elements in mycorrhiza biofertilizer bind soil particles, improve their aggregating capabilities, stabilize soil aggregates, and check leaching of important elements. Fungal elements also free the solids of the heavy metals by absorbing them and binding them in their cell walls.

As symbionts of plant roots, AMF (arbuscular mycorrhizal fungi) are critical components of soil microbial communities which influence above-ground productivity and plant community development (Sanders, Clapp, and Wiemken 1996; van der Heijden, Klironomos, Ursic *et al*. 1998), confer improved water relations (Allen and Allen 1986; Neumann and George 2004), increase nutrient uptake and stress tolerance (Lapointe and Molard 1997), and assist in stable aggregate formation and enhanced carbon and phosphorus dynamics in the rhizosphere (Coleman and Crossley 1996; Sanders, Clapp, and Wiemken 1996; Jeffries, Gianinazzi, Perotto *et al.* 2003).

In the present study, after one year of plantation, the pH of solid waste is reduced up to 7.15, and salts are also reduced in a great quantity (Table 1). Increase in organic carbon, nitrogen, phosphorus, and potassium content was also observed with each plant species used in the experiment. It might be due to improve in aggregating capabilities and reduction in leaching of important elements by mycorrhiza. Medina, Vassileva, Barea *et al.* (2006) reported that mycorrhizal treatment resulted in an increase of essential nutrients and a decrease of the contaminant metal.

The mycorrhizal colonization in amended soils had an enhancing effect for specific absorption rates of nutrients (nitrogen, phosphorus, and potassium) and a non-significant effect for the pollutant mineral studied (zinc). This selective and contrasting AM effect for nutrients and pollutant acquisition indicates the benefit of this symbiosis under contaminated conditions (Medina, Vassileva, Barea *et al*. 2006). In fact, mycorrhizal plants in amended soil have an increased amount of nutrients (nitrogen, phosphorus, and potassium) absorbed per unit of root mass (Koide 1993).

Figure 1 clearly showed the green cover on the solid dumping site after one year of plantation of four different plant species with mycorrhizal technology. All plant species got their average height and stem girth (Table 2). In an experiment, Bi, Li, Christie *et al.* (2003) reported that root colonization by AMF gave higher maize yields, higher plant uptake of most of the

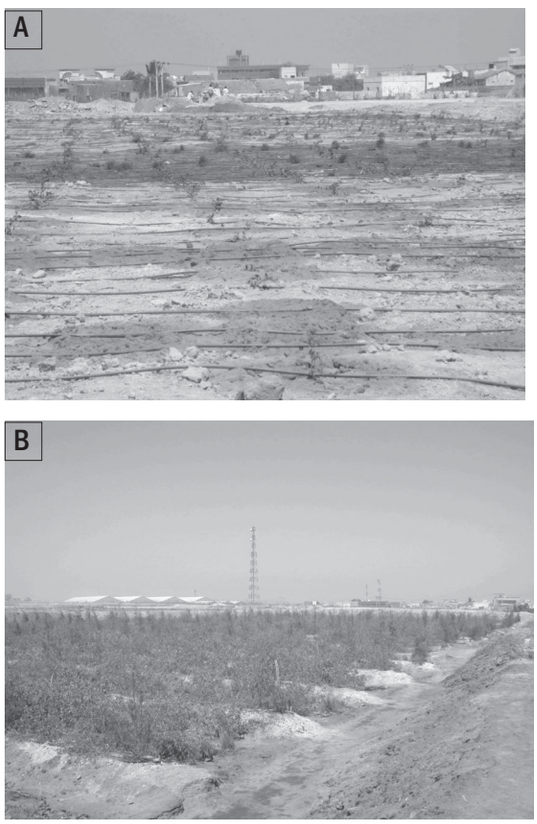

Figure 1 (A) Site at the time of plantation and (B) after completion of one year

Table 2 Average height and girth of plants grown at solid waste dumping site.

		Height			*Girth at 37 cm*		
Plant species	*Number* of plants	*Average height* (feet)	*Maximum height* (feet)	*Minimum height* (feet)	*Average girth* (cm)	*Maximum girth* (cm)	*Minimum girth* (cm)
Desibabool	14	5.3	7.4	3.10	6.5	14	2
Rambabool	5	5.2	9.0	2.11	8.2	16	4
Casurina	172	5.8	11.2	1.00	12.7	20	2
Paras pipal	438	5.4	10.2	1.10	12.0	20	3

nutrients studied, and may have protected the plants from excessive accumulation of sodium in the shoots when grown in soil overlying fly ash. Mycorrhizal colonization represents an energetic cost to the host plant in the form of carbon supplied to the mycosimbiont (Douds, Pfeffer, and Shachar-Hill 2000). This cost could be compensated by the functionality of this symbiosis since AMF absorbs photosynthates, but nutrients are supplied in return (Medina, Vassileva, Barea *et al*. 2006).

The establishment of mycorrhiza in disposed solid waste by the chemical industry may require the addition of AMF strains more suited for such an environment. selection, culture, and inoculation of soil organisms highly adapted to the types of plants, and soil conditions of a severely disturbed landscape may be an effective means for ensuring success of the re-vegetation effort.

This study indicates that successful growth of different plant species is possible in solid waste generated from chemical industries and can be improved by colonization of the plant roots by mycorrhizal fungi. Thus, remediation of areas infilled with solid waste may be possible using either undisturbed soil containing viable propagules of indigenous AMF or disturbed soil inoculated with effective strains of AMF. The fungi can assist plants in the exploitation of soil and solid waste's nutrients and may help them to resist excessive salt (sodium) accumulation. AMF may also contribute to the re-establishment of a general soil microflora, and of a sustainable agricultural system combined with the appropriate use of fertilizers.

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Recent references

The latest additions to the network's database on mycorrhiza are published here for 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.

- *Agronomy for Sustainable Development*
- *Annals of Forest Science*
- *Aquatic Botany*
- *Biocontrol Science and Technology*
- *Communications in Soil Science and Plant Analysis*
- *Environmental Pollution*
- *Experimental Agriculture*
- *Fems Microbiology Ecology*
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- *Journal of Basic Microbiology*
- *Journal of Biotechnology*
- *Journal of Forest Research*
- *Journal of Hazardous Materials*
- *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde*
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- *Mycological Progress*
- *Mycological Research*
- *Mycorrhiza*
- *New Phytologist*
- *Nuclear Instruments and Methods in Physics Research Section B-Beam Interactions with Materials and Atoms*
- *Phytochemistry*

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

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Forthcoming events

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E-mail: lpkrylova@sky.chph.ras.ru, aleshnikova@imce.ru
Website: http://www.mosbiotechworld.ru/eng/index.php

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Website: http://www.bio2007.org/index.html

24–27 June 2007
San Diego Convention Centre, San Diego, California, USA

2007 AAPS National Biotechnology Conference
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Erratum*

The introduction in the paper titled 'Effect of bio-inoculant organisms on growth and yield of Coleus forskohlii Briq.—an endangered medicinal plant' by Santosh Dharana, Laxminarayan Hegde, A K Rokhade, C P Patil, and M S Kulkarni published in Mycorrhiza News Vol. 18, No. 2. pp. 15–17, 2006 was inadvertently left out. The same is given below.

Introduction

Coleus forskohlii Briq., belonging to the family Lamiaceae, is a perennial herbaceous medicinal crop. This crop gained popularity by virtue of its exclusive constituent 'forskolin', a deterpenoid present in its dry tubers (Shah, Bhat, Bajwa, *et al.* 1980). The forskolin has shown its positive effect in glaucoma, congestive heart failure, asthma, and certain types of cancers. Due to increased demand for forskolin, the tubers were being collected from its natural stand through indiscriminate harvest, which has earned *C. forskohlii*, the status of endangered species (Vishwakarma, Tyagi, Ahmed, *et al.* 1988). Presently, organic farming plays an important role in medicinal crop cultivation. Though organic farming is lacking of clarity and depth, some beneficial microbes are reported to help and enhance soil fertility by means of recycled farm wastes. It is known that micro-organisms break complex chemicals into elemental form and supply them to the roots of the plants; in addition, organic farming reduces the building of residual (toxins) on the surface of plant leaf fruit and grain.

There are reports providing evidence that infection with mycorrhizal fungi facilitates better nutrients uptake (Adivappar 2002). *Azotobacter* sp., *Azospirillum* sp., and *Pseudomonas* sp. also appear to be better options for the facilitation of nutrient uptake. The role of individual micro-organism is well known, but their interactive role in production requires exploration. Hence, the present study was undertaken to study the effect of AMF (arbuscular mycorrhizal fungi) in the growth and yield of *C. forskohlii*.

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* The error is regretted

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