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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

Carbohydrate metabolism by mycorrhizae

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Though mechanisms leading to infection by mycorrhizal fungi on fine host roots are not fully understood, accumulation of carbohydrates and metabolites (root exudates-M-factor) of Melin (1963), in addition to some vitamins, attract the ectomycorrhizal fungal symbionts from the soil to grow on surface of roots and cause infection. It is, therefore, essential to know the processes involved in the assimilation of carbohydrates by the mycorrhizal fungi in order to understand the process of symbiosis between the plant roots and mycorrhizal fungi.

Presence of carbohydrates in mycorrhizal roots

In studies conducted at Physiologische Okologie der Pflanzen, Botanisches Institut der Universitat Tubingen, Auf de Morgenstelle 1, 7400 Tubingen, Germany, on compartmentation of carbohydrates such as sucrose, glucose, fructose, and mannose in different parts of an ectomycorrhiza established between *Picea abies* and *Amanita muscaria*, lyophilized mycorrhizae and fine roots (length 2 mm) were dissected into slices of about 0.5 mm thick. These slices represented four zones of different physiological functions. The total amount of the analysed carbohydrates was approximately 30% higher in non-mycorrhizal compared with mycorrhizal fine roots, with sucrose being the dominant sugar in both the root types. A longitudinal distinction of sucrose pools showed lowest levels in the middle parts of a mycorrhiza, which represent areas of most intense symbiotic interaction. Fine roots without fungal infection did not show longitudinal variations in sugar content (Rieger, Guttenberger, and Hampp 1992).

In studies conducted at the Research Section of Wood Chemistry, Wood Research Institute, Kyoto University, Uji, Kyoto 611, Japan, lyophilized and crushed roots of *Pinus densiflora* were extracted with water in order to investigate nutritional relations between the mycorrhizal edible fungus, *Tricholoma matsutake* and its host *P. densiflora*. Amylose, amylopectin, glucomannan, and arabinan were major components of the neutral polysaccharides; the acidic fraction consisted mainly of arabinoglucuronoxylan and homogalacturonan (Watanabe, Inaba, Nakai, *et al.* 1991).

Studies conducted at the University of Tubingen, Institute of Botany, Morgenstelle, Tubingen, Germany, on sterile in vitro and non-sterile pot experiments provided the data that gave evidence that hexoses were supplied to the fungus by the host plant (mainly glucose and fructose), and that these sugars, at least in part, controlled the development and function of ectomycorrhizae by interfering with fungal gene expression. The functionality of ectomycorrhizae is largely dependent on the ability of the host plant to supply photoassimilates to the fungus via the symbiotic interface. Any factor that reduces hexose allocation to the host–fungus interface will adversely affect ectomycorrhizae development (Hampp, Wiese, Mikolajewski, *et al.* 1999).

Production of hydrolytic enzymes by vesicular arbuscular mycorrhizal fungi/roots

By spores/hyphae of vesicular arbuscular mycorrhizal fungi

In studies conducted at the Soil Microbiology Department, Estacion Experimental del Zaidin, Prof. Albareda 1, Granada, Spain, production of hydrolytic enzymes such as pectinases, cellulases, and hemicellulases by extracts of spores of *Glomus mosseae* was observed. The possible involvement of hydrolytic enzymes in the process of root infection by *G. mosseae* in *Medicago sativa* plants grown under axenic conditions was studied. The presence of pectin or sodium pectate substrate in the rooting medium delayed the beginning of mycorrhizal infection and decreased the plateau of the infection curves. Hemicellulose substrate (locust bean) had no effect on VA (vesicular arbuscular) infection. Carboxymethyl cellulose at concentrations of 0.2% and 0.6% decreased VA infection from the beginning of the experiment (Garcia-Romera, Garcia-Garrido, Martinez-Molina, *et al*. 1990; Garcia-Romera, Martinez-Molina, and Ocampo 1988).

Further studies conducted at the above Institute showed that the spores and external mycelium of *G. mosseae* possessed a complex of pectolytic enzymes, including pectinesterase, polymethylgalacturonases, polygalacturonases, pectin, and pectate lyases. No significant differences between spores and external mycelium were found in pectin esterase, endo-polymethylgalacturonase, polymethylgalacturonase, and pectin lyase activities. However, greater polygalacturonase and lower endo-polygalacturonase and pectate lyase activities were found in the external mycelium compared to spores (Garcia, Garcia Garrido, and Ocampo 1991).

By VA mycorrhizal roots

Studies conducted at the Soil Microbiology Department, Estacion Experimental del Zaidin, Granada, Spain, showed that the type of extraction solution used influenced the pectolytic enzyme recovery from lettuce roots colonized by *G. mosseae*. The most suitable extraction buffers were 250 mM NaCl (sodium chloride) for pectin esterase and 50 mM citrate–phosphate plus polyvinyl–polypyrrolidone for polygalacturonase, endo-polygalacturonase, polymethylgalacturonase, endo-polymethylgalacturonase, pectin, and pectate lyase. The inclusion of glycine and urea to citrate–phosphate buffer decreased polygalacturonase. Mycorrhizal roots possessed more pectin esterase than endo-polymethylgalacturonase. Contents of polygalacturonase, polymethylgalacturonase, endo-polygalacturonase, pectin, and pectate lyases were similar in colonized and non-colonized roots. It was thus suggested that pectin esterase and endo-polymethylgalacturonase may participate in the process of penetration and colonization of roots by *G. mosseae* (Garcia-Romera, Garcia-Garrido, Martinez-Molina, *et al*. 1991).

In another study conducted at the above Institute, the production of pectinases in lettuce was examined during root colonization by the VAM (vesicular arbuscular mycorrhizal) fungi, *G. mosseae* and *Glomus fasciculatum*. Pectin esterase activity was higher in the inoculated plants than in the non-mycorrhizal control plants, although activity increased in the latter in 60-day-old plants. Pectin esterase activity in mycorrhizal roots remained constant. Endo-polymethylgalacturonase and polymethylgalacturonase activity peaked after 60 days and 30 days, respectively. Pectin lyase activity in lettuce and maize peaked at 30 days when inoculated with *G. mosseae*, but not until 60 days in plants inoculated with G. fasciculatum. No significant differences in endopolygalacturonase, polygalacturonase, and pectate lyase activities were observed between the VAM and control plants (Garcia-Romera, Garcia-Garrido, and Ocampo 1992).

Another study conducted at the above Institute on the measurement of cellulase activity in lettuce cv. Romana and onion cv. Babosa colonized by *G. mosseae* showed that the type of extraction solution influenced cellulase recovery. As more activity was detected in colonized plants compared with non-colonized plants, it was suggested that cellulase enzymes were involved in the colonization process (Garcia-Garrido, Garcia-Romera, Ocampo 1992).

In further studies conducted at the above Institute, XET (xyloglucan endotransglycosylase; EC 2.4.1) activity during the process of penetration and development of the AM (arbuscular mycorrhizal) fungus, *G. mosseae*, in roots of onion (*Allium cepa*, cv. Babosa) was investigated. Viscometry assays did not yield any detectable transglycosylation products. However, a radiochemical assay showed that in both mycorrhizal and non-mycorrhizal plants, XET activity decreased as the roots grew. Xyloglucan endotransglycosylase activity was higher in mycorrhizal plants than in non-mycorrhizal plants. There may be a possible role of XET associated with wall loosening in the colonization of the plant root by AM fungi (GarciaRomera, GarciaGarrido, and Ocampo 1999).

In studies conducted at the Moscow University, Moscow, *Glomus* spp. were cultured axenically on Hepper's medium to determine the xylanase activity.

Xylanase activity was highest in 30-day-old cultures of *G. fasciculatum* (0.46 U/ml) while *in G. mosseae* it was only 0.022 U/ml. Plant tissue indicators (mycorrhizal maize roots) increased the secretion of extracellular xylanases. There is thus a possible participation of xylanase secretion in the process of penetration into host roots and its effect on the host–endophyte relationship in VAM symbiosis (Shnyreva and Kulayv 1990).

In studies conducted at the National Grassland Research Institute, Nishinasuno, Tochigi, Japan, roots of onion plants colonized by *Gigaspora margarita* were treated with a digestion solution containing cellulase and pectinase for one hour at 30 °C, then homogenized with a waring blender at low speed. The internal hyphae were collected from the homogenate by centrifugation on a discontinuous gradient of Percoll, then purified further by filtration. Enzyme histochemical staining showed that the collected internal hyphae had active SDH (succinate dehydrogenase), ALP, and ACP (alkaline and acid phosphatases) (phosphoric monoester hydrolases). Specific activities (protein basis) of several enzymes in a crude extract of the internal hyphae were compared with those of axenically germinated spores of *G. margarita*. The specific activities of hexokinase, the enzymes involved in phosphate metabolism (ALP, ACP), and the TCA (tricarboxylic acid) cycle (SDH, malate dehydrogenase) were much greater in the internal hyphae than in the germinated spores. The specific activity of phosphofructokinase was similar in both. By contrast, the specific activity of glucose-6-phosphate dehydrogenase was greater in the germinated spores (Saito 1995).

In studies conducted at the Fachbererich Biologie der Philipps, Universitat Marburg, Karl von Frisch-Strasse, Marburg, Germany, mycorrhizal and non-mycorrhizal roots of *Allium schoenoprasum* were tested for activities of alpha-mannosidase, beta-glucosidase, and arabinosidase. Mannosidase activity was higher by a factor of two in mycorrhizal than in non-mycorrhizal root extracts. The apparent molecular weight of the enzyme was 152 kDa and its K_m was 1.25 mM in colonized roots and 1.85 mM in uncolonized roots. Alpha mannosidase activity was further characterized by an acid pH optimum and Zn^{2+} dependency. No significant differences could be found between mycorrhizal and non-mycorrhizal roots for beta-glucosidase and arabinosidase activities (Abrecht, Redecker, Thierfelder, *et al*. 1996).

Studies were conducted at the Utah State University, Department of Biology, Logan, UT, USA, to determine how carbon availability was a driving force in regulating location and function of arbuscules in cortical cells as arbuscules were considered to be the key site of interchange of carbon between root cells and the hyphae of arbuscular mycorrhizal fungi. Altered expression, specifically in the arbusculated cell, of genes that govern sucrose hydrolysis may create a sink for sucrose in the cells. The authors proposed a role for vacuolar invertase and cytoplasmic sucrose synthase in catalysing the intracellular hydrolysis of sucrose, thus maintaining a gradient for symplastic influx of sucrose into the arbusculated cell and establishing a gradient for hexose efflux to the apoplast for fungal utilization. VAM fungi may regulate hydrolysis of sucrose by stimulating the expression and activities of plant invertases by the production of plant hormones as well as through acidification of the arbuscular interface. It was thus speculated that altered plant defense gene expression in arbusculated cells was consistent with regulation by sugar sensing mechanisms (Blee and Anderson 1998).

Production of hydrolytic enzymes by ectomycorrhizal fungi/roots

By ectomycorrhizal fungi

Studies conducted at the Institute of Biology, Nicolaus Copernicus University, ul. Gagarina 9, Torun, Poland, showed that *Hebeloma crustuliniforme* was more cellulolytic and pectolytic than *Laccaria laccata* and *Pisolithus tinctorius*. Also, *H. crustuliniforme* and *P. tinctorius*, but not *L. laccata*, produced proteolytic enzymes. No effect of vitamins was found on the production of the cellulases, proteases, polygalacturonase, and pectate lyase enzymes by the three fungi (Dahm and Strzelczyk 1995).

Earlier studies conducted at the above Institute on cellulolytic and pectolytic activities of streptomycetes isolated from root-free soil, rhizosphere, and mycorrhizosphere of pine (*Pinus sylvestris* L.) showed that most of the streptomycetes isolated from the root-zone, but only a few of those from root-free soil, showed cellulolytic activity. A few of the root zone organisms, but none of the soil isolates, showed pectolytic activity. Root-zone isolates include those from the rhizosphere and the mycorrhizosphere (soil directly adhering to mycorrhizae plus the surface of mycorrhizae). Cellulolytic activity was generally higher in root-zone isolates than in root-free soil isolates. The isolates studied produced only endopolymethylgalacturonase; its total activity was higher in rhizosphere isolates but specific activity was higher in mycorrhizosphere organisms (Strzelczyk and Szpotanski 1989).

In studies conducted at the Department of Botany, University of Wyoming, Laramie, Wyoming, USA, simple sensitive colorimetric assays for

saprotrophic enzyme activity in ectomycorrhizal and saprotrophic basidiomycetes were derived for in vivo growth situations. These assays were developed for cellulases, phenoloxidases, phosphatases, and proteases. Enzymatic activity was visualized by placing glass fibre filter paper soaked with agar containing one of the several reaction mixtures in contact with ectomycorrhizal root systems and mycelia of saprotrophic fungi. Cellulase, along with protease and phosphatase enzyme systems, was visualized by staining residual substrate following exposure. The studies showed that cellulase and phenoloxidase activities were distributed over the entire mycelium of saprotrophic fungi tested, whereas in ectomycorrhizal fungi, greatest activity occurred near the growing hyphal front and away from ectomycorrhizae. Protease activity was greatest near the hyphal front though it was also evident in older hyphae in both ectomycorrhizal and saprotrophic fungi. Phosphatase activity was greatest near ectomycorrhizae than in extramatrical hyphae but in saprotrophs greatest activity was near the hyphal front (Miller 1993).

In studies conducted at the Department of Bacteriology and Biochemistry, Institute of Molecular and Agricultural Genetics Engineering, University of Idaho, ID, USA, carbon nutrition and production of hydrolytic and cellulolytic enzymes were investigated in four strains of *P. tinctorius*, that is, SMF, S471, S359, and S370. Glucose, mannose, and cellobiose supported rapid mycelial growth of all four strains. Fructose was utilized by SMF and S359. Of the 10 hydrolytic enzymes examined, acid alpha-galactosidase, acid phosphatase, acid esterase, and acid beta-glucosidase were found in all four strains. beta-galactosidase was only observed in strain S 359. Alpha-mannosidase, beta-mannosidase, alpha-glucosidase, beta-xylosidase, and proteinase were not detected in any of the four strains. Isoenzyme patterns of beta-glucosidase and esterase in the four strains were compared by activity staining after native gradient gel electrophoresis. The isoenzyme pattern of beta-glucosidase showed three major forms in all four strains. In addition, two more isoforms were found in strain S370. All strains shared two esterase bands, while strain S370 had three more isoforms. Tests on strain SMF indicated that acid beta-glucosidase was expressed constitutively, with increased activity in cellobiose-containing media. Under nitrogen-limiting conditions, a small amount of endoglucanase and exoglucanase activity was observed in strains SMF and S359. In S359, a high concentration of nitrogen repressed the cellulolytic activity. When the carbon source was cellobiose, greater cellulolytic activity was observed in S359; however, cellulose did not induce greater acitity in this strain (Cao and Crawford 1993).

By ectomycorrhizal roots

Studies conducted at the Escola Superior Agrana de Praganca, Praganca, Portugal, on B-glucosidase activity in mycorrhizal (with *P. tinctorius*) and non-mycorrhizal roots of *Casatnea sativa* and *Quercus rubur* determined spectrophotometrically showed that after two hours of incubation, the activity was higher in roots and leaves of mycorrhizal plants than non-mycorrhizal plants (Martins, Keller, Pais, *et al*. 1993).

Effect of soil pollutants on enzyme production by ectomycorrhizal fungi

In studies conducted at the Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA, ectomycorrhizal roots and visibly associated rhizomorphs with the roots of *Salix rotundifolia* growing in hydrocarbon – or non-hydrocarbon – impregnated soils were isolated and examined for endo-, exo-, and B-glucosidase (cellulase), and aryl hydrocarbon hydroxylase activities. These systems were also incubated in 14C-UL-cellulose and 14C-l-hexadecane and incorporation and generation of $14CO_2$ monitored. The same parameters were examined for cultured *Cenococcum graniforme* isolated from *S. rotundifolia* roots. Rhizomorph isolates from non-hydrocarbon-impregnated tundra showed general cellulase activities, typical of saprophytic fungi, while mantle tissue had lower levels of exo- and B-glucosidase activities. Both systems showed $14CO_2$ generation and incorporation paralleling cellulase activities. Only isolates from hydrocarbon–impregnated soils showed aryl hydrocarbon hydroxylase activity or hexadecane uptake. Differences between mantle and rhizomorph hyphae were not as great as those noted for cellulase activities. Cultured *C. graniforme* showed cellulase and aryl hydrocarbon hydroxylase activities more similar to rhizomorph hyphae than to mantle hyphae (Linkins and Antibus 1979).

Studies were conducted at the Department of Environmental Science and Engineering, Nanjin University, Nanjin, China, on the influence of copper, manganese, and pH on enzyme activities in ectomycorrihzal fungus, *A. muscaria*. Copper (5–25 mg/litre) and lower pH (3.0–4.0) strongly inhibited mycelial growth (DW) of *A. muscaria* but protein content was unaffected. Some enzyme activities were lower while others were maximum with increase in copper and manganese concentrations. The activities of the following enzymes were significantly correlated with fungal growth after treatment with copper: glucose-6-phosphate dehydrogenase, mannitol dehydrogenase, and trehalase. With manganese, following enzymes were significantly correlated with fungal growth: glucose-6-phosphate dehydrogenase,

mannitol dehydrogenase, and alpha-mannosidase. It was suggested that measurement of these enzyme activities might provide a useful biochemical criterion for the evaluation of the fungitoxicity of soil contaminated by copper or manganese (Kong 1995).

Effect of humus on enzyme production by ectomycorrhizal fungi

Studies were conducted at the Johannes Gutenberg University, Institute of General Botany, Mainz, Germany, on the activities of three enzymes of the carbohydrate metabolism, namely glucosephosphate isomerase, pyruvate kinase, and 6-phosphogluconate dehydrogenase, and two of the major organic acids, namely citric acid, and malic acid on mycorrhizal roots of Norway spruce (*P. abies*.). Annual mean activities of the three enzymes were equal in mycorrhizal roots of the humus and the upper mineral soil. The activities of each of the three enzymes were higher in autumn and winter than in summer. Of the various soil treatments, only soil acidification affected the activities of the three enzymes. It stimulated activities by a factor of 1.5 in mycorrhizal roots of the humus but had no effect on mycorrhizal roots from the upper mineral soil (0–5 cm). Mycorrhizal roots in the humus contained approximately 10 times more citrate and two times more malate than mycorrhizal roots from the upper mineral soil. In mycorrhizal roots from the humus, citrate and malate were of similar concentrations. In mycorrhizal roots from the upper mineral soil, malate was approximately four times more concentrated than citrate. In the humus, the citric acid concentration of mycorrhizal roots decreased under soil acidification by a factor of 1.4 while it increased under liming and compensatory liming (acid irrigation under liming) by a factor of 1.5. Malic acid concentrations increased exclusively under liming in mycorrhizal roots of the humus by a factor of 1.3 (Nowotny, Schwanz, and Rothe 1998).

Carbohydrate assimilation in mycorrhizal roots

In studies conducted at the Biochemistry Department, University of Georgia, Athens, USA, it was demonstrated that fructose-2, 6-biphosphate regulated mycorrhizal tree root pyrophosphate-dependent phosphofructokinase. It also activated the breakdown of sucrose in tree sink tissues such as roots and terminal buds. Sucrose breakdown via the sucrose synthase pathway was dependent on pyrophosphate, and is strongly activated by fructose-2, 6-biphosphate (Sung, Xy, Mustardy, *et al.* 1989).

The studies conducted at the Universite de Nancy I, Laboratoire de Physiologie Vegetal et Forestiere, B.P. 239, 54506 Vandoeuvre les Nancy, Cedex, France, showed that the process of mycorrhization in spruce (*P. abies*) trees was characterized by changes in the capacities of several enzymes linked to carbon and nitrogen metabolism. The carbohydrate breakdown was shifted from the glycolytic to the pentose phosphate pathway. Moreover, there was occurrence of a cyanide resistance electron transport in mitochondria from mycorrhizal roots (Namysl, Chalot, Dell, *et al.* 1990).

In studies conducted at the Umist, Department of Biomol Science, Manchester, Lancs, England, isolates of ectomycorrhizal fungi, *Suillus variegatus* and *P. tinctorius* along with a *Cortinarius* sp. and the white rot *Phanerochaete chrysosporium* were examined for their ability to oxidize carbohydrates to their corresponding lactones and to excrete the H_2O_2 (hydrogen peroxide) produced thereby. All except *P. chrysosporium* were found to express cellobiose oxidase (cellobiose dehydrogenase, EC 1.1.19.88) and glucose oxidase (beta-D-glucose:oxygen 1-oxidoreductase, EC 1.1.3.4) when grown on cellobiose and glucose, respectively. Production of extracellular H_2O_2 was visualized during growth on both substrates using ABTS as the chromogen. According to the Fenton reaction, H_2O_2 will react with hydrated or chelated Fe_2 in the environment to produce hydroxyl (Fenton) radicals, HO. Mycelial extracts from each of the mycorrhizal fungi produced HO. In the presence of cellobiose and Fe_2, presumably mediated by H_2O_2 produced by cellobiose oxidase activity in the extracts, conditions favourable to HO production were shown to exist in Modified Melin–Norkrans medium (Burke and Cairney 1998).

Synthesis of mycorrhiza-specific carbohydrates by ectomycorrhizal fungi/roots

By ectomycorrhizal roots

In studies conducted at the Abteilung Pflanzenphysiol., Botany Institute, University of Basel, Basel, Switzerland, excised mycorrhizas on fine roots of *P. abies* trees, grouped according to state of development and integrity, were tested for their ability to produce fungal and plant-specific soluble carbohydrates from 14C-labelled glucose. Trehalose, a fungal-specific disaccharide, was the principal sugar formed. A strong correlation was found between the rate of trehalose synthesis and the proportion of intact, turgid, young, and vigorous mycorrhizas. Measurement of the rate of trehalose synthesis is, therefore, recommended by the authors as a more objective alternative to morphological description in

evaluation of mycorrhizal vitality in fine roots (Niederer, Pankow, and Wiemken 1988).

In studies conducted at the Division des Pepinieres, MER, 200 Chemin Ste-Foy, Quebec, Canada, soluble sugars of balsam fir ectomycorrhizal and non-mycorrhizal root apexes were analysed by gas chromatography. The pattern of soluble sugars belonging to mono-, di- and trisaccharides was different for mycorrhizal and non-mycorrhizal roots. Trehalose and mannitol were observed almost exclusively in ectomycorrhizal roots that also contained less glucose, fructose, and sucrose as compared to non-mycorrhizal root apexes. Several sugars varied markedly during the growth season and this was the case with mannitol in the brown ectomycorrhizae. Sucrose and raffinose varied in ectomycorrhizal and non-mycorrhizal roots. Also, marked differences were observed in the mannitol contents of the different types of ectomycorrhizae (Langlois and Fortin 1981).

In the Studies conducted at the Institute of Physiol. Botany, University of Uppsala, Uppsala, Sweden, glucose-6-phosphate dehydrogenase, hexakinase, and phosphoglucose-isomerase extracted from roots of *P. sylvestris* L. or *Fagus orientalis* were not inhibited by mannitol at concentrations up to 0.5 M in contrast to previous reports for these enzymes from *Fagus sylvatica*. The K_m-values for the carbohydrate substrates were the same in the presence or absence of mannitol (50 mM) (Jirjis, Ramstedt, and Soderhall 1986).

In the studies were conducted at the Physiologische Okologie der Pflanzen, Universitat Tubingen, Auf der Morgenstelle, Tubingen, Germany, on the non-reducing disaccharide trehalose (-D-glucopyranosyl (1-1)-D-glucopyranoside), which is often involved in the metabolism of the microsymbiont in symbiotic associations. Trehalose has been suggested to be toxic for certain plants and plant cell structures, as long as they do not possess a specific enzyme, trehalase, for the hydrolytic cleavage of trehalose. In this context, the content of soluble sugars and sugar alcohols in roots, ectomycorrhizas, and vegetative mycelium of the ectomycorrhizal partners, Norway spruce (*P. abies*) and fly agaric (*A. muscaria*), was analysed, both enzymatically and by different chromatographic techniques such as anion exchange chromatography with pulsed amperometric detection. With the latter method, this polysaccharide could be shown to constitute a major soluble fungus-specific carbohydrate, both in vegetative mycelium of *A. muscaria* and in mycorrhizas of *A. muscaria* and *P. abies*. In addition, a trehalase activity was found in the roots of plants grown on MMN (modified Melin Norkrans)-medium supplemented with trehalose, which is interesting with respect to the potential toxicity of trehalose. Plants grown on glucose instead of trehalose did also show trehalase activity, suggesting that the enzyme was not induced. The acidic pH-optimum supports the assumption that the activity is possibly localized in an acidic compartment like the cell wall, where it could hydrolyse trehalose leaching from the microsymbiont and convert it to glucose that is available for both partners (Wallenda, Guttenberger, and Hampp 1993).

In further studies conducted at the above University, PEPC (phosphoenolpyruvate carboxylase) activity, F26BP (a regulator of sucrose synthesis), starch, sucrose, glucose, and fructose were determined in needles and mycorrhizas/roots of Norway spruce (*P. abies*) seedlings, either non-mycorrhizal or mycorrhizal, with *Laccaria bicolor*. Nutrient supply, especially nitrogen supply, is of great importance for the regulation of carbon partitioning between carbohydrates and amino acids, and PEPC is an important enzyme connecting carbohydrate and amino acid metabolism in plants. The enzyme produces carbon skeletons for the synthesis of amino acids and thus reduces synthesis of carbohydrates, especially of sucrose. Plants of Norway spruce were grown in a semi-hydroponic cultivation system under different nutrient regimes such as balanced nutrients, nutrients without magnesium, nutrients without nitrogen and phosphorus, with nitrogen but without phosphorus, without nitrogen but with phosphorus, and with both nitrogen and phosphorus. In needles, specific activity of PEPC was correlated with a high content of F26BP, a regulator that inhibits sucrose synthesis. In parallel, a reduced starch content in nitrogen plus treatments supports the assumption of an increased demand for carbon skeletons for amino acid synthesis. Decreased PEPC activities in the phosphorus plus treatments are, so far, difficult to interpret. Mycorrhization had no effect on PEPC activity, F26BP, and starch content in needles, whereas PEPC activity was lower in mycorrhizal than in non-mycorrhizal roots. This effect was especially high in the +B/–P treatment where enzymes of the fungus instead of PEPC probably fulfil anaplerotic functions. Sucrose, glucose, and fructose contents were generally higher in non-mycorrhizal fine roots than in mycorrhizas. This indicates that the fungus depletes the roots of soluble sugars possibly by converting them into fungus-specific carbohydrates (trehalose, mannitol, glycogen) (Wingler, Einig, Schaeffer, *et al.* 1993)

By ectomycorrhizal fungi

In studies conducted at the Physiologische Okologie der Pflanzen, Universitat Tubingen, Tubingen, Germany, trehalase activity was analysed from the mycelium of the ectomycorrhizal fungus, *A. muscaria*, growing in liquid culture or on agar plates, and from

mycorrhizas for a better understanding of carbon metabolism as trehalose, a non-reducing disaccharide, is a major storage compound in most fungi. Trehalase activity was found in both the culture medium and in mycelium extracts. The excreted trehalase was purified to apparent homogeneity by anion-exchange chromatography, gel filtration, and preparative gel electrophoresis. The identified enzyme belonged to the group of acid trehalases. The apparent molecular mass of the native enzyme was estimated to be 165 kDa by gel filtration and 135 kDa by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Isoelectrofocusing indicated an isoelectric point (pl) of approximately 3.7, which was typical for acid trehalases. The enzyme was highly specific for its substrate trehalose, with an apparent K_m of 0.38 mM. Validamycin and sucrose act as competitive inhibitors with K_i values of 45 mM and 15 mM, respectively. The activity of acid trehalase excreted into the growth medium was independent of the carbon source (glucose or trehalose), revealing that the enzyme was not regulated by its substrate trehalose. Nevertheless, fungal hyphae grown in the absence of an external carbon source showed enhanced enzyme excretion. The biochemical characteristics of the trehalase activity measured in the mycelium were in the same range as those determined for the excreted enzyme. The enzyme was localized in the cell wall and its activity was strongly decreased in ectomycorrhizas (Wisser, Guttenberger, Hampp, *et al.* 2000).

In studies conducted at the Laboratoire de Microbiologies, Institut National de la Recherche Agronomique, Champenoux, Seichamps, France, carbohydrate and amino acid synthesis and accumulation were studied in several ectomycorrhizal ascomycetes (*Cenococcum graniforme*, *Sphaerosporella brunnea*) and basidiomycetes (*L. laccata*, *H. crustuliniforme,* and *Piloderma croceum*) in order to examine possible common features of primary metabolism in ectomycorrhizal fungi. Carbon-13 NMR (nuclear magnetic resonance) spectroscopy was used to follow the metabolism of (1–13C) glucose: its conversion to carbohydrates and free amino acids.
The kind of carbohydrates and free amino acids synthesized from glucose was remarkably similar throughout the studied fungi. Incorporation of the 13C label resulted in the accumulation of large amounts of labelled mannitol, alanine, glutamine, and glutamate. Comparison of the pathways used to synthesize these compounds revealed that the ectomycorrhizal fungi possess identical primary metabolism pathways. The most relevant information resulting from this investigations is (1) the occurrence of the mannitol cycle, (2) a large part of the trehalose is synthesized after cycling of glucose-carbon through the pentose phosphate pathway or mannitol cycle, (3) a major sink of TCA cycle intermediates is the formation of glutamate, glutamine, and alanine, and (4) CO_2 fixation, via pyruvate carboxylase and phosphoenolpyruvate carboxykinase, furnishes high pools of amino acids (Martin, Ramstedt, and Soderhall 1987).

In studies conducted at the Department of Physiol. Botany, University of Uppsala, Uppsala, Sweden, the compounds synthesized and accumulated during glucose utilization by *Piloderma croceum* were identified and characterized by the use of NMR spectroscopy. The studies showed that mannitol comprised 54% of the total observable natural abundance of 13C and the remaining carbon was accumulated in fatty acids. During (1–13C) glucose assimilation, the majority of the 13C label was incorporated into mannitol, while trehalose was labelled to a much lesser extent. Scrambling of the 13C label in trehalose suggests that part of the glucose was cycled through a metabolically active mannitol pool. The labelling pattern of carbohydrates and the occurrence of the necessary enzymes indicated that the mannitol cycle was operative in *P. croceum* (Ramstedt, Martin, and Soderhall 1990).

Synthesis of mycorrhiza specific carbohydrates by VA-mycorrhizal fungi/roots

By VAM roots

Studies were conducted at the Institute Coltivazioni Arboree dell'Universita', Via P. Giuria 15, Torino, Italy, on the occurrence of trehalose in VAM fungi and VAM roots. Trehalose, a disaccharide present in many microorganisms, may have the role of a reserve carbohydrate, or it may be a part of stress metabolism. Trehalose is one of the main carbohydrates in ectomycorrhizal fungi.

Using gas chromatography, the presence of trehalose was investigated in the roots of different plant species (leek, white clover, soybean, *Tagetes tenuifolia*), infected with *G. mosseae* or *G. versiforme*, and in sporocarps of these fungal species. Significant amounts of trehalose were found in the mycorrhizal roots of some host/fungus combinations, while non-mycorrhizal roots contained negligible amounts of the disaccharide, which can be accounted for by bacterial contamination. Trehalose was also present in fungal sporocarps. Further studies were conducted on the activity of the main enzymes of trehalose metabolism as they are present in yeast, that is, trehalose-6-P synthase and neutral trehalase. Non-mycorrhizal roots showed no activity of both enzymes, while mycorrhizal roots had activities that were comparable to those of yeast extracts. The fact that the occurrence of trehalose and the activities of enzymes related to its

metabolism were not evenly present in all mycorrhizal roots suggests that in VAM fungi, trehalose was not only used as a long-time reserve carbohydrate, but that it may play other role in the fungal carbon metabolism (Schubert, Wyss, and Wiemken 1990).

In further studies at the above University, trehalose was found as the main soluble carbohydrate in the sporocarps of *Glomus versiforme*. Trehalose was also present in mycorrhizal roots, whereas in non-mycorrhizal roots, it was absent or detected only in traces. In soybean plants inoculated with *G. mosseae* and grown under natural light or dark regimen and given 0 or 5 mM phosphorus as KH_2PO_4 (potassium dihydrogen phosphate) and K_2HPO_4 (potassium monohydrogen phosphate), phosphorus fertilizer and light deprivation reduced root mycorrhizal infection. The trehalose content of mycorrhizal roots increased concurrently with the course of fungal infection, and decreased both with phosphorus fertilizer and light deprivation. The content of total soluble carbohydrates, on the contrary, decreased only with light deprivation (Schubert, Wyss, and Wiemken 1992).

Further studies at the above University showed trehalase activity in the root extracts of leek (*Allium porrum L.*) and soybean (Glycine max L.Merr.). This activity was inhibited by phloridzin and was several times higher than the activity of general α-glucosidase. The activity had an acidic optimum. Trehalase activity in extracts of sporocarps and extraradical mycelium of the VAM fungus, *G. mosseae*, was higher than that in root extracts and had an optimum at pH 7. Following inoculation with *G. mosseae*, trehalase activity increased in mycorrhizal roots above the levels observed in non-mycorrhizal roots. Irrespective of fungal colonization, root trehalase activity increased in the presence of Mg_2, decreased in the presence of Mn_2 and Zn_2, and was unaffected by Na_2EDTA (ethylene diamine tetra acetic acid) (Schubert and Wyss 1995).

By VAM spores

Studies were conducted at the United States Department of Agriculture, Agriculture Research Service, ERRC, Philadelphia, PA, USA, to detect mannitol and trehalose by analysing a very large number of spores, the stage in the lifecycle of VAM fungi most likely to contain storage compounds. Natural abundance 13C NMR spectra were obtained for spores of three species of VAM fungi, that is, *Glomus intraradices*, *G. etunicatum*, and *G. margarita*. A clear observation of the resonances corresponding to trehalose has been found in *G. etunicatum* and *G. margarita* spectra. HPLC (high performance liquid chromatography) analyses of the methanol/water extracts of the spores supported these observations and indicated that *G. intraradices* spores also contained low levels of trehalose. Further support for the presence of trehalose was obtained after treating the spore extracts with trehalase, when only glucose was detected by HPLC. Trehalose was found to constitute 0.06%, 1.5%, and 1.07% (w/w) of the spore dry weight for *G. intraradices*, *G. etunicatum*, and *G. margarita*, respectively. For the three species, only trace amounts of other sugars were detected (sucrose, glycerol, and fructose for *G. margarita*, and glycerol for *G. etunicatum* and *G. intraradices*). Preliminary results from an in vivo NMR time course experiment indicated that trehalose was readily utilized during spore germination. These results demonstrate for the first time that trehalose, in addition to lipid, is a major form of carbon storage in VAM fungi (Becard, Doner, Rolin, *et al.* 1990)

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Research finding papers

Arbuscular mycorrhizal fungal association of cowpea (*Vigna unguiculata* (L.) Walp) grown in coastal lowland and inland fields of Mangalore *taluka*

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Cowpea, *Vigna unguiculata* (L.) Walp, is commonly grown as a rabi crop in the low-lying fields of coastal areas and inland fields of Mangalore. Mangalore is one of the *talukas* of Dakshina Kannada, which is located between 12° 29' 36" N and 13° 49' 22" N latitudes, and 74° 37' 24" E and 75° 41' 00" E longitudes. The coastal low-lying fields of Mangalore periodically get inundated by the backwaters, as a result of which they suffer from high soil salinity. Salinity of the agricultural area increases as it nears the coastal lands (Aliasgharzadeh, Saleh Rastin, Towfighi, *et al*. 2001). Salinity of the soil may affect the distribution and abundance of mycorrhizal fungi. So, attempts were made to study the distribution of AMF (arbuscular mycorrhizal fungi) spores in the rhizosphere of three commonly grown cultivars of cowpea plants, and to evaluate the root colonization by AMF in the coastal lowland fields and inland fields of Mangalore in relation to some physico-chemical properties of soil.

Materials and methods

Three cultivars of *V. unguiculata* (L.) Walp, cv. GC3 (Gujarat cowpea), cv. MBSL (Mangalore brown spotted local) and cv. MVL (Mangalore vegetable local), were planted in small field plots, each of 100 m^2 (square metres) size, in coastal lowland fields of Adam Kudru and inland fields of Vamanjoor during the month of January 2004. From each plot, five different plants were selected for sampling after fourth and eighth week of planting. Samples of rhizosphere soils (0–20 cm deep) and young primary roots were collected. Five sets of roots and rhizosphere soil samples per plot were thoroughly mixed, and a composite was taken for analysis of colonization.

The roots were excised, washed carefully, cut into 1-cm segments, stained and examined for AMF as described by Philips and Hayman (1970) and Koske and Gemma (1989). The total percentage of root colonization was determined by the root slide techniques, as described by Giovannetti and Mosse (1980). AMF propagules were collected from the soil by wet seiving and decanting method and counted (Gerdemann and Nicolson 1963). The AMF spores were identified with the help of relevant literature (Morton 1988; Schenk and Perez 1990). Moisture content, pH, and EC (electrical conductivity) of rhizospheric soil were determined within two to three hours of the collection of soil sample. Soil pH and EC were measured after dilution with distilled water (1:1 v/v). Na (Sodium), K (potassium), total N (nitrogen), available P (phosphorus), and OC (organic carbon) of the soil samples were measured by following the methods described in soil chemical analysis by Jackson (1973).

Correlation analysis was performed for the evaluation of the relationships between different soil properties and number of spores or percentage root length colonized.

Results and discussion

The data (Table 1) suggests that the pH of the soil of coastal lowland fields was higher than that of the inland fields. The EC of the soil of inland field did not vary much but the EC of the coastal lowland field showed a significant increase from fourth week to the eighth week. An increase in the Na content in the coastal lowland field was observed. A significant positive correlation ($r = +0.811$, $P < 0.05$) was observed between salinity and Na content of the soil. Per cent root colonization in the coastal lowland fields was strongly negatively correlated to EC ($r = -0.865$, $P < 0.05$) and Na ($r = -0.766$, $P < 0.05$). The spore density was also negatively correlated to EC and Na content; but it was not significant.

In both the coastal lowland fields and inland fields, no significant correlation was found between P, K, OC, N, and spore number/root colonization. The spore density in the rhizospheric soils of all the three cultivars of coastal lowland fields was less compared to that of inland fields. In both coastal and lowland fields, the spore density increased significantly during the eighth week when compared to the fourth week. The same trend was observed in the percentage of root length colonized. The percentage of root length colonized was higher in GC3 compared

Table 1 Physico-chemical characteristics of rhizosphere soils, and percentage of arbuscular mycorrhizal fungi colonization and spore number in the three cultivars of cowpea

	Fourth week						*Eighth week*					
	GC3		*MBSL*		*MVL*		*GC3*		*MBSL*		*MVL*	
	CLF	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*
PH	7.18	6.61	7.32	6.81	7.11	6.89	7.19	6.77	7.21	6.87	6.95	6.85
EC ds/m	0.16	0.06	0.11	0.06	0.11	0.07	0.44	0.06	0.44	0.06	0.40	0.07
Moist %	17	19	17	19	15	19	16	19	15	19	15	20
Na (mg/g)	0.320	0.122	0.349	0.126	0.395	0.123	0.430	0.122	0.428	0.127	0.438	0.122
K (mg/g)	0.108	0.076	0.108	0.086	0.106	0.082	0.113	0.090	0.120	0.068	0.118	0.074
P (mg/g)	2.57	1.17	2.21	1.59	2.56	1.45	2.62	1.12	2.35	1.13	2.68	1.23
OC(%)	0.736	0.562	0.739	0.583	0.715	0.584	0.742	0.432	0.733	0.515	0.723	0.573
N (mg/g)	14.94	15.28	14.54	15.43	17.24	17.17	14.11	16.05	14.98	15.32	16.35	18.13
Spore number /l00 Soil	80	95	70	90	75	85	95	120	85	116	82	105
Percentage of root colonization	50	78	45	68	49	66	50	85	47	77	53	73

CLF –coastal lowland field; IF – inland field; EC – electrical conductivity; Na – sodium, P – phosphorus; K – potassium; N – sodium; MBSL – Mangalore brown spotted local; MVL – Mangalore vegetable local; GC3 – Gujarat cowpea; OC– oeganic carbon

to that of other cultivars in both coastal and inland fields.

The AMF spores isolated in the rhizospheric soils of both coastal and inland fields is given in Table 2. The most predominant AMF in the coastal lowland fields were *Glomus mosseae* (Nicol and Gerd.) Gerdemann and Troppe, *Glomus aggregatum* Schenck and Smith emend Koske and *Scutellospora nigra* (Reanead) Walker and Sanders.

The higher salinity levels of coastal lowland fields compared to the inland fields may be due to the intrusion of seawater to land (Mohandas and Marimuthu 2002).

The results of the present investigation indicate that salinity of soil in the coastal fields increased significantly from fourth week to eighth week. The same results were also reported by Chandrashekar and Sandhyarani (1995). Lower root

Table 2 Arbuscular mycorrhizal fungi spores isolated from the soils of study sites

AMF spores	*Fourth week*						*Eighth week*					
	GC3		*MBSL*		*MVL*		*GC3*		*MBSL*		*MVL*	
	CLF	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*	*CLF*	*IF*
Acaulospora scrobiculata Trappe	+			+		+				+		+
Glomus microcarpum Tul and Tul					+		+		+		+	
Glomus monosporum Gerdemann and Trappe		+		+				+		+		
Glomus multicaule Gerdamann and Bakshi						+						+
Glomus mosseae (Nicol and Gerd.) Gerdemann and Trappe	+		+	+	+		+		+		+	
Scutellospora nigra (Rednead) Walker & Sanders	+		+		+		+		+		+	
Gigaspora sp. (1)				+								

AMF – arbuscular mycorrhizal fungi; MBSL – Mangalore brown spotted local; MVL – Mangalore vegetable local; GC3 – Gujarat cowpea; CLF –coastal lowland field; IF – inland field; GC3–Gujarat cowpea

colonization in all the cowpea cultivars of coastal lowland fields with increase in salinity corroborates with the results of Aliasgharzadeh, Saleh Rastin, Towfighi *et al*. (2001). They also stated a negative correlation between Na^+ content of soil and root colonization in onion, alfalfa, wheat, and barley. Juniper and Abbot (1993) and Abdi and Dube (2003) also reported that with the rise in Na^+ content in the soil, there was a gradual decline in per cent colonization and spore density. Wang, Uu, Lin, *et al*. (2004) stated that AMF propagules were less in saline soils of the yellow river delta.

The colonization of root systems by *Endogone* sp. was generally low during the early stages of root growth, but the proportion of roots colonized subsequently increased to maximum during growth (Reddy, Rachel, and Reddy 1997, Sutton 1973). In the present investigation also, the per cent root colonization in the cowpea cultivars of both coastal lowland fields and inland fields increased from the fourth week to the eighth week. Beena, Raviraja, and Shridhar (2000) and Kulkarni, Raviraja, and Shridhar (1997) have reported that *Glomus mosseae* was one of the dominant AMF species in the coastal sand dunes of west coast of India. In our study, it was found that *G. mosseae* and *Scutellospora nigra* were common in the rhizospheric soils of all the three cultivars grown in coastal lowland fields. These AMF of native saline soils may have higher tolerance for salinity and may be useful as potential biofertilizers for saline soils when mass multiplied and introduced into soil.

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Fly-ash amended soil improves colonization and spore count of arbuscular mycorrhizal fungal cultures

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Introduction

Relative efficacy of mycorrhizal fungi is a measure of their ability to benefit plant growth or otherwise increase its health, either directly through increased uptake of nutrients or indirectly by improving soil conditions (Abbott and Robson 1991). There are three prerequisites for obtaining dual cultures. These are an efficient VAM (vesicular arbuscular mycorrhizal) fungus, a suitable host, and a suitable substrate. Efficient VAM fungal isolates could be selected for various desirable traits, multiplied, and inoculated onto crops, but the conditions that would allow these organisms to express their full potential will have to be determined. Selection for efficient indigenous AM (arbuscular mycorrhizal) fungal communities could take place with proper management practice. Fly ash, a particulate residue of thermal power plants, affects the growth, productivity, and reproductive abilities of a number of plants (Satyanarayan and Pushpalata 1991; Saquib and Khan 1999). As a soil pollutant, fly ash is highly alkaline and rich in salts (Adriano, Page, Elscewi, *et al.* 1980). A number of researchers (Clark 1993; Manz 1998) have compiled extensive data on the production and utilization of coal fly-ash. Studies show that with biological intervention, one can grow commercially important plant species in fly ash covered areas (Adholeya 2000). VAM fungi have been used as bioremediation agents (Leyval, Turnau, and Haselwandler 1997), and biofertilizers for agricultural, horticultural, and silvicultural plant species in polluted area (Anonymous 2002). VAM helps in binding the fine particles of ash and arrests the uptake of heavy metals by host plants. Sarangi and Mishra (1998) have reported that when mixed with soil, coal-fired fly ash improved soil conductivity, available phosphorous, organic carbon, and carbon/nitrogen ratio, and also increased the soil pH. Therefore, we conducted experiments to study the effect of fly ash mixing on the development and spore production of VAM fungi in plant rhizosphere.

Materials and methods

The experiments were conducted with four VAM fungi, that is, *Acaulospora denticulata*, *Gigaspora albida*, *Glomus albidum*, *Sclerocystis sinuosa*, inhabiting either *bajra*, maize, sorghum, or sudan grass grown in three different types of substrates, that is, sand:soil (1:1), sand:soil (1:1) + 1% fly ash (wt/wt), and sand:soil (1:1) + 4% fly ash (wt/wt). Fly ash was obtained from the ash pond (Arkha) of Feroze Gandhi Unchahar Thermal Power Project, NTPC (National Thermal Power Corporation), Unchahar, Rae Bareli. Host plants were grown in earthen pots of 30-cm diameter containing 10 kg soil substrate (8 kg substrate + 2 kg soil-based VAM fungi culture having 70 or more spores/100 g of soil) for multiplication. There were 15 replicate pots for each VAM fungi, host, and substrate combination. Control plants were raised in unsterilized substrate, with naturally occurring VAM fungi (like sand:soil [1:1] without ash, sand:soil [1:1] + 1% fly ash, sand:soil [1:1] + 4% fly ash).

Different growth parameters like root and shoot length, root and shoot dry weight, per cent root colonization, and spore count in each substrate were recorded 30 and 60 DAS (days after sowing) to determine suitable, preferred host and substrate for the multiplication of a particular VAM fungus.

Results

The results given here are the average of 15 replicates for each treatment.

Sand:soil (1:1) substrate

The plants that were inoculated with VAM fungi in the substrate sand:soil (1:1) showed the enhancement in growth parameters over control. *Bajra* was found to be the best host, with 62% root colonization in this soil mixture. The spore count recorded with *bajra* roots was 160 spores/100 g of soil 60 DAS. This was followed by maize with 58% root colonization, producing 155 spores/100 g of soil, and sudan grass with 53% root colonization and 130 spores /100 g of soil (Table 1).

Dry shoot weight and dry root weight were also recorded to be maximum in *bajra*, followed by maize, sudan grass, and sorghum when compared with control (Table 2). The *bajra* plants, when inoculated with *G. albidum*, produced 7.3 g dry root weight/plant and 35.8 g dry shoot weight/plant. The same VAM

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Table 1 Mycorrhizal colonization and production of spores by different host-vesicular arbuscular mycorrhizal fungi combinations as influenced by variations in fly ash contents of soil at the age of 60 days.

		Colonization percentage			*Spore count/100 g soil*		
Host	*VAM Fungi*	*No fly ash*	*1% fly ash*	*4% fly ash*	*No fly ash*	*1% fly ash*	*4% fly ash*
Bajra	*Glomus albidum*	62	62	55	160	175	90
	Gigaspora albida	65	68	50	112	120	84
	Acaulospora denticulata	52	52	42	95	105	80
	Sclerocystis sinuosa	48	35	45	68	60	50
	*Control	21	22	14	58	55	25
Maize	*G. albidum*	58	60	56	155	165	80
	G. albida	57	55	48	154	161	75
	A. denticulata	50	48	43	151	160	60
	S. sinuosa	25	22	40	57	60	55
	Control	18	22	22	50	50	30
Sudan grass	*G. albidum*	53	52	55	130	142	60
	G. albida	55	56	48	120	125	50
	A. denticulata	50	51	45	145	146	48
	S. sinuosa	40	40	42	63	65	45
	Control	23	25	20	40	50	28
Sorghum	*G. albidum*	53	50	52	125	128	60
	G. albida	51	52	50	126	126	51
	A. denticulata	48	50	46	100	115	49
	S. sinuosa	36	40	40	45	48	46
	Control	20	22	18	38	45	26

*Control (naturally mycorrhizal)

Table 2 Changes in dry biomass (g/plants) of hosts growing in different levels of fly-ash-amended soil due to VAM (vesicular arbuscular mycorrhizal) fungi 60 DAS (days after sowing)

VAM fungi	*Host*	*No fly ash*		*1% fly ash*		*4% fly ash*	
		Root (wt)	*Shoot* (wt)	*Root* (wt)	*Shoot* (wt)	*Root* (wt)	*Shoot* (wt)
Glomus albidum	*Bajra*	7.3	35.8	7.8	36.2	5.6	28.5
Gigaspora albida		7.1	33.6	7.3	33.8	5.5	28
Acaulospora denticulata		6.6	32.2	7	33	5.6	26.2
Sclerocystis sinuosa		4	30.4	6.8	31.6	5.2	22
*Control		3.6	15.6	5.1	25.2	3.9	15.2
G. albidum	Maize	6.9	33.8	7.1	35.2	4.9	27
G. albida		6.3	32.6	7	33.6	5	26.3
A. denticulata		6.2	32.3	6.4	30.4	4.1	25.1
S. sinuosa		5.6	32.6	6	30.2	3.9	25
Control		3.8	14.8	4.6	23.8	2.8	14.2
G. albidum	Sudan grass	6.6	33.6	7	34.2	4.5	25.8
G. albida		6.5	33.3	6.9	34	4	25
A. denticulata		6.2	32.6	6.9	32.8	3.8	24.6
S. sinuosa		5.8	32	5.7	32.1	3.5	23
Control		4	14	4.2	22.6	2.3	12.6
G. albidum	Sorghum	5.9	32.8	6.2	33.1	4	25.6
G. albida		5.2	32.6	6	33	3.8	24.2
A. denticulata		4.8	32	5.6	32.6	3.5	22.1
S. sinuosa		4.6	32.2	5.3	32.2	2.8	20
Control		3.8	13.6	4	20.2	2.1	11.6

*Control (naturally mycorrhizal)

fungi in maize plant produced 6.9 g dry root weight/plant and 33.8 g shoot dry weight/plant. More or less similar trends were recorded with other VAM fungi namely, *S. sinuosa* and *A. denticulata* (Table 2).

Sand:soil (1:1) + 1% fly ash

VAM-fungi-inoculated plants raised in the sand:soil (1:1) + 1% fly ash showed the maximum growth response in comparison to control and those grown without fly ash in sand:soil (1:1) substrate. In this substrate also, *bajra* plants were found to be the best host. After two months, the mycorrhizal colonization and the spore count in rhizosphere soil became 62% and 175 spores/100 g, respectively. This was followed by maize with 60% root colonization, producing 165 spores/100 g of soil and sudan grass with 52% root colonization and 142 spores/100 g of soil. Though there were some variations in the mycorrhizal colonization, these were not significant as compared to sand:soil (1:1) substrate (Table 1).

The *bajra* plants produced maximum dry root weight (7.8 g/plant) and dry shoot weight (36.2 g/plant) with *G. albidum* (Table 2).

Sand:soil (1:1) + 4% fly ash

Mycorrhizal plants in the substrate sand:soil (1:1) + 4% fly ash showed retarded growth responses in comparison to control and those plants that were grown in unamended or 1% fly ash amended soil. In this substrate, root colonization percentage and spore count were adversely affected when the fly ash percentage was raised to 4%. Maximum root colonization and spore count were, respectively, 55% and 90 spores/100 g of soil in *bajra* at the age of 60 days. This was followed by maize with 56% root colonization and 80 spores/100 g of soil and sudan grass with 55% root colonization (60 spores/100 g of soil) (Table 1).

Root weight and shoot weight of test plants were also reduced at 4% level of fly ash addition (Table 2).

Discussion

In the present study, the mycorrhizal colonization and spore count were highest (68% and 175 spore/100 g of soil, respectively) in the substrate composed of sand:soil (1:1) + 1% fly ash. The effect of fly ash amendment at three concentrations (10, 20, 30g fly ash/kg soil) on the infectivity and efficacy of *Glomus aggregatum* in Pigeon pea (*Cajanus cajan*) and Chick pea (*Cicer aritenum*) was studied by Reddy and Garampalli (2002). They found that the intensity of VAM colonization in both the crops gradually decreased with the increase of fly ash in soil, but increase in the total dry weight was more or less same in both the plants. Garampalli, Deene, and Reddy (2005) again studied the effect of 10, 20, and 30 g fly ash/kg amended soil on *G. aggregatum–C. cajan* symbiosis and reported complete suppression of vesicles and arbuscules at 30 g fly ash/kg soil level. However, fly ash amendment without VAM inoculum was also found to enhance the growth of plants as compared to control plants (without fly ash and VAM inoculum).

Best effects on biomass production were noticed in VAM-inoculated plants grown in soil mixture containing 1% fly ash. The plant height and dry weight increased when any one of the four VAM fungi was inoculated along with 1% fly ash. The enrichment of pond ash with organic materials enhanced its capacity to retain moisture and support plant growth. Also, organic material decomposition produces acids that bind heavy metal ions reducing their mobility and thereby reducing the surface run-off and groundwater pollution. Possibly, VAM symbiosis increases the capacity of plants to trap and accumulate phosphate as it is released by some other rhizosphere microorganisms.

The role of VAM fungus *Glomus mosseae* and nodulating *Rhizobium* sp. on the establishment, growth, and yield of black gram variety T9 in enriched, abandoned ash ponds of the Neyveli Thermal Power Plant has been reported by Sheela and Sundaram (2003). They found that plant height, dry weight, and pod yield increased when *G. mosseae* was co-inoculated along with Rhizobium sp. The nitrogen and phosphorus contents of grains were also increased. Better yield in cucumber was obtained in ash ponds on incorporation of various organic amendments (Manivannan and Baskaran 2001). AM benefit plants by improving the supply of nutrients, especially phosphorus and minerals such as zinc, copper, sulphur, potassium, and calcium (Cooper and Tinker 1978), and the plant supplies the fungus with photosynthetic sugars (Varma and Schuepp 1995). Presence of potentially harmful trace elements and toxic heavy metals like lead, tin, cadmium, arsenic, antimony, mercury, chromium, aluminium, copper, and zinc in fly ash is reported. Increased tolerance of mycorrhizal plants to toxic heavy metal concentrations in the soil makes mycorrhiza significant. Fly ash from power plants can be used to reclaim problematic soils and can thus reduce disposal problems. It is used as a nutrient in agriculture or horticulture and also as a liming agent in acidic agricultural soils (Plank, Martens, and Hallock 1975; Sheela and Sundaram 2003). Martens (1970) used it to counter nutrient deficiency or increase nutrient uptake by crops. Presumably, fly ash–soil mix laden with VAM fungal spores can be a good ameliorative solution for reclaiming acidic soil.

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Response of mango rootstocks inoculated with arbuscular mycorrhizal fungi and bioformulations to soft wood grafting

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Introduction

Mango tree is an important subtropical fruit tree grown in India on an area of 1.6 million hectares with a production of 10.8 MT (million tonnes) (Anonymous 2004). There is immense variation in mango seedlings raised even from a single tree due to the highly cross-pollinated nature of mango. Although seedling trees produce heavy crop, fruit size and quality are inferior and do not fetch a good return in the market. The seedling trees have a long juvenile period and more vigorous growth habits, which creates difficulties in taking plant protection measures and harvesting of their fruits. The fruits of seedling trees do not mature in one stroke, thereby affecting their marketing. Keeping in view these disadvantages of seedling trees, and to obtain uniformity in plant performance, the present study was undertaken to find out the effect of AM (arbuscular mycorrhizal) fungi and bioformulations on stem girth and softwood grafting.

Material and methods

The experiment was set up adopting a factorial, completely randomized block design with eight treatments, having four sub-treatments and three replications to study the effect of different AM fungi and bioformulations on softwood grafting of container-grown mango seedlings using a potting mixture of red soil, sand, and FYM (farm yard manure) in a 2:1:2 ratio. Three AM fungal species – *Glomus fasciculatum, Gigaspora margarita,* and *Acaulospora laevis* – individually and in combinations, and uninoculated formed the treatments, whereas panchagavya, amrit pani, microbial consortia, and control formed the sub-treatments. AM fungal inoculation was done by placing 5 g of inoculum uniformly at a depth of 5 cm after putting a thin layer of soil on the inoculum. Mango stones obtained from a single lot of uniform size and shape from a processing unit were dipped in 3% bioformulations for one hour and were then covered with soil. Four-month-old, uniform, healthy, disease- and pest-free seedlings were selected from the seedlings raised for pot culture experiment for germination, and were used for softwood grafting (wedge method). The scions were collected from the shoots of selected alphonso mother plants, which were defoliated 10 days prior to the grafting operation in order to activate the terminal buds. The cured scions were collected and the softwood grafting by wedge method was performed.

Results and discussion

The perusal of the data from Table 1 indicates that the plants inoculated with *G. margarita* recorded significantly higher stem girth of 7.593 mm, whereas triple inoculation recorded significantly lower stem girth (6.910 mm).

All the bioformulations had recorded significantly higher stem girth compared to control. Among the three bioformulations, mango rootstocks inoculated with microbial consortia recorded the highest stem girth of 7.470 mm followed by panchagavya with 7.266 mm and amrit pani with 7.203 mm. Significantly lower stem girth was recorded in control (7.110 mm).

Interaction effects of AM fungi and bioformulations on stem girth were found to be significant. Highest stem girth was recorded in seedlings inoculated with *G. margarita* along with microbial consortia (7.953 mm), which was statistically on par with treatment combination consisting of *G. fasciculatum* along with microbial consortia (7.880 mm). Significantly lower values for stem girth were recorded in treatment with triple inoculation of *G. fasciculatum*, *G. margarita*, and *A. laevis* without bioformulations (6.867 mm). The highest stem girth observed with the inoculation of *G. fasciculatum* and microbial consortia could be due to their higher efficiency/host preference as evidenced by higher spore count and per cent root colonization (Table 2) as against uninoculated control.

The perusal of the data presented in Table 2 indicates that the influence of AM fungi on graft-take after three months of grafting was significant. The plants inoculated with *G. margarita* recorded the highest graft success of 64.16%, which was statistically on par with *G. fasciculatum* (60.00%) and *A. laevis* (59.16%). Significantly lower success of graft-take was noticed in triple inoculation of *G. fasciculatum*, *G. margarita*, and *A. laevis* (50.83%), which was statistically similar to that of dual inoculation of *G. margarita* and *A. laevis* (52.50%).

Table 1 Effect of arbuscular mycorrhizal fungi and bioformulations on stem girth and microbial parameters

Treatment	*Stem girth* (mm)								
	Pancha gavya	*Amrit pani*	*Microbial consortia*	*Control*	*Mean*	*Per cent root colonization*		*Spore count*	
T1 – *Glomus fasciculatum*	7.373	7.433	7.880	7.333	7.505	93.66		814.41	
T2 – *Gigaspora margarita*	7.523	7.440	7.953	7.463	7.593	91.25		807.58	
T3 – *Acaulospora laevis*	7.357	7.357	7.707	7.367	7.447	88.91		797.58	
T4 – T1 + T3	7.197	7.163	7.440	6.940	7.185	77.41		628.83	
T5 – T1 + T2	7.180	7.147	7.240	6.973	7.135	78.25		629.58	
T6 – T2 + T3	7.090	7.113	7.130	6.980	7.078	76.75		627.83	
T7 – T1 + T2 + T3	6.953	6.897	6.923	6.867	6.910	71.16		609.08	
T8 – Control	7.457	7.073	7.490	6.953	7.243	43.91		259.83	
Mean	**7.266**	**7.203**	**7.470**	**7.110**		**77.66**		**646.84**	
For comparing the means of	S.Em±		CD at 5%			S.Em±	C.D. at 5%	S.Em±	C.D. at 5%
Main (T)	0.025		0.070			0.691	1.942	1.345	3.780
Sub (S)	0.018		0.051			–	–	–	–
T x S	0.050		0.141			–	–	–	–

Table 2 Effect of arbuscular mycorrhizal fungi and bioformulations on graft-take and sprout length of mango grafts

Treatment	*Graft success (%)*					*Graft survival (%)*					*Sprout length (%)*				
	Pancha-gavya	*Amrit pani*	*Microbial consortia*	*Control*	*Mean*	*Pancha-gavya*	*Amrit pani*	*Microbial consortia*	*Control*	*Mean*	*Pancha-gavya*	*Amrit pani*	*Microbial consortia*	*Control*	*Mean*
T1– *Glomus fasciculatum*	63.33 (–52.77)	56.66 (–48.84)	63.33 (–52.86)	56.66 (–48.84)	60 (–50.83)	33.33 (–35.21)	33.33 (–35.21)	36.66 (–37.22)	30 (–33.21)	33.33 (–35.21)	7.64	7.52	7.59	7.44	7.55
T2 – *Gigaspora margarita*	63.366 (–52.77)	60 (–50.77)	73.33 (–59)	60 (–50.85)	64.16 (–53.35)	43.33 (–41.15)	36.66 (–37.22)	50 (–45)	33.33 (–35.21)	40.83 (–39.64)	8.86	8.78	8.96	8.67	8.81
T3 – *Acaulospora laevis*	60 (–50.85)	60 (–50.77)	63.66 (–52.77)	53.33 (–46.92)	59.16 (–50.33)	36.66 (–37.14)	33.33 (–35.21)	40 (–39.14)	30 (–33.21)	34.91 (–36.17)	8.4	8.3	8.5	8.03	8.31
T4 – T1 + T3	56.66 (–48.84)	56.66 (–48.84)	60 (–50.77)	53.33 (–46.92)	56.66 (–48.84)	33.33 (–35.21)	26.66 (–30.99)	36.66 (–37.22)	26.66 (–30.99)	30.82 (–33.6)	6.95	6.85	6.99	6.76	6.89
T5 – T1 + T2	60 (–50.85)	60 (–50.77)	60 (–50.85)	50 (–45)	57.5 (–49.36)	30 (–33)	30 (–33)	30 (–33)	23.33 (–28.77)	28.33 (–31.94)	6.63	6.72	6.81	6.52	6.67
T6 – T2 + T3	53.33 (–46.92)	50 (–45)	56.66 (–48.84)	50 (–45)	52.5 (–46.44)	30 (–33)	26.66 (–30.99)	30 (–33)	23.33 (–28.77)	27.5 (–31.44)	6.49	6.4	6.6	6.3	6.44
T7 – T1 + T2 + T3	50 (–45)	50 (–45)	53.33 (–46.92)	50 (–44.91)	50.83 (–45.46)	30 (–33)	26.66 (–30.99)	30 (–33)	26.66 (–30.99)	28.33 (–31.99)	6.36	6.29	6.56	6.19	6.35
T8 – Control	60 (–50.77)	60 (–50.85)	63.33 (–52.77)	43.33 (–41.07)	56.66 (–48.86)	36.66 (–37.22)	36.66 (–37.14)	40 (–39.14)	23.33 (–28.77)	34.16 (–35.57)	7.59	7.44	7.64	6.03	7.18
Mean	58.33 (–49.85)	56.66 (–48.85)	61.66 (–51.85)	52.08 (–46.19)		34.16 (–35.61)	31.2 (–33.84)	36.66 (–37.09)	27.03 (–31.24)		7.37	7.29	7.46	6.99	
For comparing the means of	S.Em±		CD at 5%			S.Em±		CD at 5%			S.Em±		CD at 5%		
Main (T)	1.177		3.31			1.191		3.347			0.012		0.034		
Sub (S)	0.833		2.341			0.842		2.367			0.009		0.025		
T x S	1.177		NS			1.191		NS			0.024		0.067		

Figures in parenthesis indicate arcsine value. NS – non-significant

Among the bioformulations, microbial consortia (61.66%) and panchagavya (58.33%) recorded significantly higher per cent success of graft-take when compared to amrit pani (56.66%) and control (52.08%). However, the effects of microbial consortia and panchagavya were statistically on par with each other. Significantly lower success was noticed in control when compared to bioformulations.

Highest per cent survival of grafts was observed in *G. margarita* (40.83%), which was significantly superior than other AM fungal inoculation and control. Dual inoculation of *G. margarita* and *A. laevis* recorded significantly lower survival (27.50%), which was statistically on par with dual inoculation of *G. fasciculatum* and *G. margarita* (28.33%), triple inoculation of *G. fasciculatum*, *G. margarita*, and *A. laevis* (28.33%), and dual inoculation of *G. fasciculatum* and *A. laevis* (30.82%). Among the bioformulations, microbial consortia (36.66%) and panchagavya (34.16%) were superior.

Among the AM fungi, plants inoculated with *G. margarita* recorded significantly highest sprout length of 8.81 cm, when compared to other AM fungal inoculation and uninoculated control. There was a significant increase in sprout length with the application of microbial consortia to the *G. margarita* inoculated grafts (8.96 cm). Significantly lower sprout length was observed with triple inoculation of *G. fasciculatum*, *G. margarita*, and *A. laevis* with 6.35 cm and in absolute control (6.03 cm).

In the present investigation, when wedge grafting was performed on three-month-old seedlings, the stem girth of AM fungi and bioformulation-inoculated rootstocks was significantly thicker than the control rootstocks. Better stem girth at the time of grafting might have influenced the graft success and survival percentage (Table 2). The effects of AM fungi and bioformulations on plants are manifested in a multiple ways, such as improving seedling emergence, root development, and plant biomass through a direct means or by reducing the damage caused by soil-borne pathogens, fixing atmospheric nitrogen, translocating phosphorus, synthesis and secreting of plant growth regulators like IAA (indole acetic acid), producing siderophores, phosphorus sobubilization, production of hormones, enzymes, and so on (Subbarao 1995).

Hence, it can be concluded from the present investigation that *G. margarita* and microbial consortia are the best AM fungal and bioformulation combinations for alphonso mango seedlings to have higher graft success and survival percentage, and increased sprout length.

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New approaches

Rapid and reliable DNA extraction techniques

Two improved DNA extraction techniques from trypan-blue-stained root fragments were developed by Ishii S and Loynachan T E (2004) and compared for rapid and reliable analysis (*Mycorrhiza* **14**: 271–275). In method A, 1 cm of trypan-blue-stained mycorrhizal root fragments were individually isolated, crushed by bead beating, and purified with Chelex-100 (Bio-Rad). In method B, DNA extraction was carried out using an ultra clean microbial DNA isolation kit (Mo Bio Laboratories). DNA was extracted from the mycorrhizal roots of four plant species, quantified by UV (ultraviolet) absorbance and PCR (polymerase chain reaction)-amplified with primers specific to arbuscular mycorrhizal fungi. Although PCR inhibitors might still exist when using method A, appropriate dilution and employment of nested PCR overcame this problem. Method B removed PCR inhibitors but sometimes, depending on the mycorrhizal colonization within the root fragments, it also required nested PCR.

In conclusion, both methods enabled the authors to handle many samples in a short time. Method B provided greater reliability while method A provided better cost performance. Both techniques can be useful for PCR-based applications to identify species and estimate species composition after measuring mycorrhizal colonization rates with trypan-blue staining.

Effect of various plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungi combinations on four different wheat varieties

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One key biotechnological goal is to use combined inoculation of selected rhizospheric microorganisms to minimize fertilizer application and to maximize plant growth and nutrition (Barea, Andrade, Biaciotto, *et al.* 1998; Probanza, Mateos, Lucas, *et al.* 2001). Reports from the literature confirm that selected combinations of microbial inocula enhance the positive effect achieved by each microbial group considered separately (Toro, Azcón, and Barea 1997, 1998). Also, free-living bacteria produce certain stimulatory metabolites that enhance plant development and growth, particularly when associated with AM fungi (Azcon 1993). Within microbial species, the wide genetic variation explains the high potential of the microorganisms to adapt to different environments. Therefore, it is necessary to develop specific host–strain combinations of both bacteria and AM (arbuscular mycorrhizal) fungi with high effectiveness under a wide range of experimental conditions (Medina, Probanza, Gutierrez Manero, *et al.* 2003). Thus, these suggestions may be taken into account while offering the AM-fungus-based bio-inoculants for sustainable plant production.

A microcosm experiment was set up to study the role of the interaction between AMF (arbuscular mycorrhizal fungi) and PGPR (plant-growth-promoting rhizobacteria). The interaction of 5 AMF and 11 PGPRs was studied in four hexaploid varieties of wheat with respect to mycorrhiza formation, wheat growth response, and plant nutrient acquisition. The microcosm assemblies were arranged in a completely randomized design with four replicates per treatment. All the assemblies were kept on raised benches in greenhouse (Figures 1 and 2). The plants were watered to a moisture level of approximately 60% of the water-holding capacity and were grown in a greenhouse at 25 ± 5 ^{0}C. Half-strength Hoagland's nutrient solution (Hoagland and Arnon 1938) was provided to the plants at fortnightly interval.

Treatments

Five AMF, 11 PGPR, and four hexaploid wheat

Hexaploid wheat

- PBW 343
- HD 2687
- UP 2338
- Kundan

Arbuscular mycorrhizal fungi

(**Source** CMCC, TERI)

- A
- B
- C
- D
- E

Plant-growth-promoting rhizobacteria

(**Source** GVBPAUT, Pantnagar)

- PSB-1
- PSB-3
- PSB-19
- PSB-34
- PSB-51
- PSB-62
- PSB-63
- PSB-109
- PSB-117
- PSB-12096
- PSB-A2

Plants were grown for four months and height was measured. The plants were then harvested and dried to a constant weight at 70 ^{0}C. Uptake of macronutrients (nitrogen and phosphorus) and micronutrients (iron, copper, cobalt, molybdenum, and zinc) was studied in the plants.

Figure 1 Microcosm experimental set-up

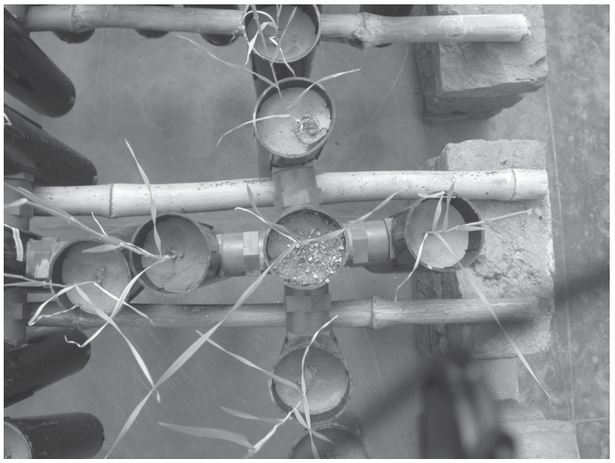

Figure 2 Five assemblies interconnected but separated by a fine mesh, which allows only hyphae and not roots to cross the assembly

Results

The data presented in Tables 1–6 indicates that all inoculated plants of wheat irrespective of variety and AMF inoculum exhibited higher grain dry weight, and increased height and nutrient uptake when compared to uninoculated plants. However, the extent of increase varies with the type of inoculation and the variety. The wheat plants inoculated with AMF alone and combined with mycorrhizal fungi showed higher nutrient-uptake than inoculation with PGPR alone. The magnitude of PGPR and AMF inoculation varies due to type variety. The PGPRs, PSB-117, PSB-62 and PSB-12092 with E and A, when co-inoculated, produced highest nutrient uptake in wheat plants.

Table 1 Comparison of plant dry weight (g) in different wheat varieties inoculated with various combinations of AMF and PGPRs (the data represents the mean value of three plants)

AMF type	*Wheat variety*	*Control*	*Only AMF*	*Only PSB*	*AMF +PSB –109*	*AMF +PSB –19*	*AMF +PSB –51*	*AMF+ PSB –3*	*AMF+ PSB –A2*	*AMF+ PSB –62*	*AMF+ PSB –63*	*AMF +PSB 12092*	*AMF +PSB –117*	*AMF +PSB –34*	*AMF +PSB –1*
No AMF	UP 2338	–	–	–	1.14	1.27	1.31	1.23	1.29	1.08	1.59	1.15	1.16	1.03	1.17
	PBW 343	–	–	–	1.29	1.12	1.23	1.43	1.35	1.14	1.18	1.63	1.14	1.28	1.25
	HD 2687	–	–	–	1.23	1.07	1.21	1.25	1.37	1.02	1.05	1.64	1.13	1.22	1.3
	Kundan	–	–	–	1.01	1.26	1.12	1.19	1.12	1.16	1.29	1.18	1.02	1.03	1.05
A	UP 2338	0.94	1.69	1.95	1.41	1.31	1.17	1.02	1.27	2	1.09	1.2	2.39	1.62	1.29
	PBW 343	1.48	1.42	1.84	1.64	1.6	1.83	1.08	1.72	1.52	1.78	1.51	1.51	1.57	1.66
	HD 2687	1.12	1.53	1.22	1.43	1.56	1.43	1.4	1.4	1.36	1.39	1.43	1.44	1.45	1.46
	Kundan	1.15	1.61	1.56	1.45	1.45	1.4	1.51	1.52	1.55	1.61	1.62	1.57	1.58	1.6
B	UP 2338	1.07	1.44	1.48	1.56	1.46	1.03	2.82	1.1	1.33	1.39	1.38	1.36	0.8	0.88
	PBW 343	1.38	1.37	1.19	1.29	1.12	1.37	1.55	1.13	1.18	1.39	0.91	1.2	1.04	1.52
	HD 2687	1.2	1.43	1.33	1.42	1.31	1.3	1.33	1.42	1.38	1.34	1.29	1.33	1.44	1.4
	Kundan	1.31	1.47	1.44	1.29	1.34	1.41	1.44	1.43	1.37	1.39	1.44	1.42	1.32	1.29
C	UP 2338	0.99	1.39	1.06	1.07	1.23	1.01	0.57	0.97	0.96	1.03	1.24	1	0.96	1.01
	PBW 343	0.95	0.96	1.6	1.72	1.06	1.58	1.51	1.27	1.45	1.72	1.39	1.31	0.91	1.17
	HD 2687	1.02	1.26	1.12	1.42	1.45	1.48	1.52	1.45	1.51	1.43	1.33	1.39	1.51	1.34
	Kundan	1.04	1.61	1.34	1.45	1.44	1.51	1.52	1.44	1.23	1.42	1.22	1.32	1.44	1.46
D	UP 2338	0.85	1.13	0.87	1.32	0.79	0.92	1.03	1.02	1.22	0.95	1.64	1.33	1.12	0.94
	PBW 343	0.84	0.88	1.05	0.76	0.6	0.99	1.2	0.89	0.93	1.1	1.07	0.81	0.91	1.12
	HD 2687	0.93	1.21	1.23	1.42	1.12	1.08	1.25	1.23	1.31	1.42	1.22	0.99	1.05	1.09
	Kundan	0.98	1.42	1.04	1.34	1.23	1.22	1.34	0.97	1.22	1.32	1.42	1.33	1.3	1.4
E	UP 2338	1.29	0.99	1.1	1.21	1.04	0.96	1.15	1.53	0.92	1.29	1.57	0.97	1.23	0.81
	PBW 343	1.1	1.95	0.99	1.38	1.1	1.32	1.42	1.96	1.07	1.73	1.05	1.96	1.63	1.02
	HD 2687	1.2	1.52	1.34	1.45	1.33	1.34	1.46	1.51	1.37	1.61	1.56	1.45	1.52	1.43
	Kundan	1.22	1.43	1.35	1.38	1.29	1.41	1.39	1.22	1.4	1.34	1.39	1.32	1.41	1.44

AMF – arbuscular mycorrhizal fungi; PGPR – plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

Table 2 Per cent increase in phosphorus uptake in different wheat varieties inoculated with various combinations of AMF and PGPRs over non-inoculated plants (the data represents the mean value of three plants)

AMF type	Wheat variety	Only AMF	Only PSB	AMF +PSB –109	AMF +PSB –19	AMF +PSB –51	AMF +PSB –3	AMF +PSB –A2	AMF +PSB –62	AMF +PSB –63	AMF +PSB 12092	AMF +PSB –117	AMF +PSB –34	AMF +PSB –1
A	UP 2338	4	10	12	10	8	10	25	24	11	29	5	12	10
	PBW 343	80	65	51	20	40	55	41	40	30	45	40	34	58
	HD 2687	72	30	40	19	23	40	30	52	50	40	46	30	15
	Kundan	73	20	20	25	28	30	28	42	20	30	32	20	20
B	UP 2338	10	8	14	12	8	12	11	28	4	30	25	19	8
	PBW 343	75	43	50	62	65	73	63	78	50	70	71	57	62
	HD 2687	3	2	6	7	2	4	6	18	12	10	15	5	6
	Kundan	20	10	22	12	6	18	21	28	8	25	26	12	18
C	UP 2338	12	6	17	15	13	23	4	26	6	25	31	10	11
	PBW 343	70	60	50	55	61	54	61	65	51	60	64	12	17
	HD 2687	10	12	12	11	17	13	10	31	15	30	10	12	11
	Kundan	35	30	41	24	42	45	33	41	23	40	25	32	30
D	UP 2338	50	40	51	45	36	40	39	50	42	52	51	38	20
	PBW 343	12	10	12	44	32	40	33	30	23	41	37	12	10
	HD 2687	15	20	14	18	20	21	22	30	12	34	41	22	21
	Kundan	45	44	31	30	19	23	21	50	14	51	50	20	12
E	UP 2338	30	20	25	20	23	29	30	42	38	39	41	20	21
	PBW 343	45	40	41	35	22	42	38	42	30	46	43	32	30
	HD 2687	82	65	70	65	45	71	76	91	80	87	80	29	41
	Kundan	35	30	41	40	31	30	20	48	40	35	41	40	39

AMF - arbuscular mycorrhizal fungi; PGPR - plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

Table 3 Per cent increase/decrease in iron uptake in different wheat varieties inoculated with various combinations of AMF and PGPRs over non-inoculated plants (the data represents the mean value of three plants)

AMF type	Wheat variety	Only AMF	Only PSB	AMF +PSB –109	AMF +PSB –19	AMF +PSB –51	AMF +PSB –3	AMF +PSB –A2	AMF +PSB –62	AMF +PSB –63	AMF +PSB 12092	AMF +PSB –117	AMF +PSB –34	AMF +PSB –1
A	UP 2338	5	6	7	4	5	8	10	15	6	19	25	6	5
	PBW 343	20	15	22	17	6	12	15	29	10	31	30	10	15
	HD 2687	38	20	22	10	12	15	17	45	32	41	40	15	20
	Kundan	20	10	15	8	12	8	9	25	10	16	25	12	10
B	UP 2338	1	4	19	11	12	10	5	22	2	21	25	10	8
	PBW 343	2	5	10	8	6	6	5	15	10	15	19	9	9
	HD 2687	6	8	10	4	4	4	6	21	11	18	20	10	10
	Kundan	12	6	8	7	6	8	9	11	10	20	21	7	6
C	UP 2338	2	6	12	10	9	8	5	25	19	21	30	10	9
	PBW 343	9	12	11	14	2	3	8	20	10	23	31	19	8
	HD 2687	2	10	9	11	10	7	9	17	11	20	24	8	9
	Kundan	12	8	7	6	7	6	10	23	10	25	29	6	10
D	UP 2338	25	20	22	20	7	5	5	6	6	23	20	9	10
	PBW 343	–2	4	34	31	12	6	4	6	33	15	21	10	4
	HD 2687	23	10	45	28	10	8	8	12	24	35	31	40	5
	Kundan	12	21	14	19	20	12	6	12	23	34	10	25	7
E	UP 2338	42	9	8	17	22	9	4	13	32	54	41	32	8
	PBW 343	42	10	10	12	12	10	7	23	44	24	21	10	12
	HD 2687	30	15	12	15	10	8	23	6	12	46	10	20	17
	Kundan	25	10	15	21	10	9	2	26	38	45	28	15	16

AMF – arbuscular mycorrhizal fungi; PGPR - plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

Table 4 Per cent increase/decrease in copper uptake in different wheat varieties inoculated with various combinations of AMF and PGPRs over non-inoculated plants (the data represents the mean value of three plants)

AMF type	Wheat variety	Only AMF	Only PSB	AMF +PSB –109	AMF +PSB –19	AMF +PSB –51	AMF +PSB –3	AMF +PSB –A2	AMF +PSB –62	AMF +PSB –63	AMF +PSB 12092	AMF +PSB –117	AMF +PSB –34	AMF +PSB –1
A	UP 2338	10	12	12	10	15	20	34	45	22	43	40	10	10
	PBW 343	22	21	31	12	16	31	30	48	18	32	30	7	14
	HD 2687	41	36	30	15	27	30	21	26	32	30	32	5	4
	Kundan	34	23	21	25	41	25	24	21	16	21	20	18	9
B	UP 2338	4	12	4	23	21	23	5	32	12	34	21	13	8
	PBW 343	5	12	35	23	31	3	12	12	5	23	22	11	6
	HD 2687	6	14	45	6	21	3	4	23	3	34	12	13	5
	Kundan	5	13	3	6	2	5	6	15	6	13	10	18	9
C	UP 2338	6	12	22	34	5	7	12	12	2	4	10	22	12
	PBW 343	5	13	12	33	23	13	21	24	4	24	11	22	12
	HD 2687	3	5	24	4	13	12	22	21	5	43	20	12	4
	Kundan	3	8	5	23	22	10	21	32	23	13	33	12	3
D	UP 2338	23	2	5	23	24	24	53	35	24	43	23	13	2
	PBW 343	2	16	22	45	32	32	12	13	34	21	13	34	12
	HD 2687	3	4	33	21	21	23	12	15	15	13	10	10	12
	Kundan	32	13	34	43	34	21	23	43	21	23	11	13	14
E	UP 2338	11	13	13	24	12	21	34	43	13	42	23	13	23
	PBW 343	23	43	43	44	13	11	13	32	14	35	12	12	23
	HD 2687	24	13	13	12	13	32	21	43	32	33	32	20	33
	Kundan	23	15	21	23	33	21	23	24	32	11	23	23	23

AMF – arbuscular mycorrhizal fungi; PGPR - plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

Table 5 Per cent increase/decrease in molybdenum uptake in different wheat varieties inoculated with various combinations of AMF and PGPRs over non-inoculated plants (the data represents the mean value of three plants)

AMF type	Wheat variety	Only AMF	Only PSB	AMF +PSB –109	AMF +PSB –19	AMF +PSB –51	AMF +PSB –3	AMF +PSB –A2	AMF +PSB –62	AMF +PSB –63	AMF +PSB 12092	AMF +PSB –117	AMF +PSB –34	AMF +PSB –1
A	UP 2338	30	24	23	23	13	56	43	41	67	42	53	13	14
	PBW 343	50	24	24	24	43	24	21	34	53	13	45	26	26
	HD 2687	70	26	24	34	42	34	22	43	13	13	14	13	42
	Kundan	50	32	13	45	35	53	23	34	24	45	45	24	43
B	UP 2338	15	43	12	12	22	21	11	21	21	42	34	23	32
	PBW 343	15	21	32	43	34	32	12	32	24	11	21	44	22
	HD 2687	10	23	34	32	21	12	42	13	14	23	34	32	21
	Kundan	20	24	24	23	23	13	12	23	22	13	23	23	34
C	UP 2338	10	23	23	23	33	11	12	44	32	32	22	23	21
	PBW 343	20	34	23	32	33	23	32	33	22	34	14	12	30
	HD 2687	12	32	23	13	24	11	33	22	34	23	15	24	14
	Kundan	30	23	32	23	22	10	23	34	11	33	35	12	16
D	UP 2338	55	45	23	34	13	36	23	12	10	11	10	33	20
	PBW 343	15	4	5	13	4	23	30	10	23	34	12	30	23
	HD 2687	40	32	36	45	45	22	32	12	23	13	16	23	10
	Kundan	20	34	56	12	24	20	12	13	26	18	21	20	15
E	UP 2338	61	65	27	32	35	30	17	38	34	34	13	23	24
	PBW 343	30	23	12	23	24	34	15	23	43	12	34	21	10
	HD 2687	50	45	51	26	43	56	13	12	33	32	32	34	24
	Kundan	35	44	25	31	35	44	11	33	23	34	30	17	13

AMF – arbuscular mycorrhizal fungi; PGPR - plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

Table 6 Per cent increase/decrease in cobalt uptake in different wheat varieties inoculated with various combinations of AMF and PGPRs over non-inoculated plants (the data represents the mean value of three plants)

AMF type	Wheat variety	Only AMF	Only PSB	AMF +PSB –109	AMF +PSB –19	AMF +PSB –51	AMF +PSB –3	AMF +PSB –A2	AMF +PSB –62	AMF +PSB –63	AMF +PSB 12092	AMF +PSB –117	AMF +PSB –34	AMF +PSB –1
A	UP 2338	20	21	32	44	22	34	44	23	43	42	32	23	32
	PBW 343	30	22	24	34	34	43	33	23	43	32	34	43	34
	HD 2687	45	21	35	45	34	45	45	43	21	32	13	10	24
	Kundan	40	21	23	35	11	34	13	23	23	23	23	34	34
B	UP 2338	10	12	37	24	45	27	13	20	12	23	16	31	12
	PBW 343	8	15	16	33	13	21	22	21	32	34	13	17	10
	HD 2687	10	54	24	23	28	18	23	25	12	23	20	7	23
	Kundan	12	10	12	12	34	17	11	33	13	31	23	6	10
C	UP 2338	12	12	4	24	25	8	4	6	23	34	23	13	11
	PBW 343	20	15	6	13	17	5	32	24	41	35	45	29	24
	HD 2687	8	4	8	32	4	6	34	45	40	34	14	31	21
	Kundan	35	35	8	15	6	5	8	12	18	31	30	21	32
D	UP 2338	40	12	27	24	6	8	21	32	10	15	12	26	24
	PBW 343	2	1	36	9	32	6	32	30	18	10	45	46	24
	HD 2687	30	17	13	7	5	6	23	34	35	24	13	24	22
	Kundan	15	20	24	23	23	25	15	11	32	12	12	17	23
E	UP 2338	25	32	23	33	39	32	29	34	8	9	34	27	14
	PBW 343	24	18	13	35	46	20	21	24	8	10	12	18	34
	HD 2687	20	24	43	21	17	18	12	13	9	17	15	27	25
	Kundan	12	18	17	30	14	32	13	23	26	9	34	17	26

AMF – arbuscular mycorrhizal fungi; PGPR - plant-growth-promoting rhizobacteria; PSB – phosphate-solubilizing bacteria

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Recent references

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

The Mycorrhiza Network will be pleased to supply any of the available documents to bonafide researchers at a nominal charge. This list consists of papers from the following journals.

- *Agronomy for Sustainable Development*
- *Acta Societatis Botanicorum Poloniae*
- *Advances in Applied Microbiology*
- *Agricultural and Forest Meteorology*
- *Applied Soil Ecology*
- *Biology and Biochemistry*
- *Brazilian Journal of Microbiology*
- *Canadian Journal of Botany-Revue Canadienne De Botanique*
- *Chemosphere*
- *Current Genetics*
- *Current Science*
- *Forest Ecology and Management*
- *Journal of Arid Environments*
- *Journal of Sustainable Agriculture*
- *Journal of The Faculty of Agriculture Kyushu University*
- *Journal of Thermal Biology*
- *Journal of Tropical Forest Science*
- *Letters In Applied Microbiology*
- *Mycorrhiza*
- *Phytochemistry*
- *Plant and Soil*
- *Soil Biology & Biochemistry*

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

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos (address of the first author or of the corresponding author, marked with an asterisk)
Ahulu E M, Gollotte A, Gianinazzi-Pearson V, Nonaka M*. 2006	**Cooccurring plants forming distinct arbuscular mycorrhizal morphologies harbor similar AM fungal species** *Mycorrhiza* **17**(1): 37–49 [Niigata University, Faculty of Agriculture, Soil Sciences Laboratory, 8050, Ikarashi 2, Niigata 9502181, Japan]
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Conferences, congresses, seminars, symposia, and workshops

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10–14 June 2007

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Bogor, **Indonesia**
17–21 July 2007

Second IMA (Indonesian Mycorrhiza Association) National Mycorrhiza Congress

Website http://www.mycorrhizas.org/files/20070222_AMI2007.pdf

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21–27 July 2007

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Second International Rhizosphere Conference - Rhizosphere 2
Le Corum Convention Centre, Montpellier, France

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