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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, was established in 1974 as the Tata Energy Research Institute. While initially, TERI's focus was mainly on documentation and information dissemination, research in the fields of energy, environment, and sustainable development was initiated towards the end of 1982. The genesis of these activities lay in TERI's firm belief that efficient utilization of energy, sustainable use of natural resources, large-scale adoption of renewable energy technologies, and reduction of all forms of waste would move the process of development towards the goal of sustainability.

The Biotechnology and Management of Bioresources Division

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

The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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

Contents

Assessment of a metabolically active VAM infection by vital staining methods

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

The extent of VAM infection in plant roots is usually measured, in terms of infected root length as a percentage of total root length, made visible by non-vital stains like trypan blue or chlorozol black. These stains, however, do not differentiate between active and inactive or dead cells of the fungal hyphae. In order to have a clear understanding of the dynamics of VAM infection and the development of fungal hyphae in roots and in soil, use of vital stains is necessary. A vital stain stains only the living cells so that metabolically active VAM fungal hyphae in roots as well as in soil can be detected and quantified. Such stains determine the activity of different fungal enzymes: NBT (Nitroblue tetrazolium) is used to determine the activity of succinate dehydrogenase found in mycorrhizal fungi. FDA (Fluorescein diacetate) is used to determine the activity of esterases in VAM fungal hyphae. Vital staining methods have also been used to study the progress of infection and the effects of fungicides, light and other environmental factors, and soil phosphorus levels on the development of VAM infection (Sukarno and Dickson 1992).

Methods of staining and extraction of enzymes from roots

In studies on enzyme staining conducted at the Soil Science Department, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, Australia, NBT was used to determine the activity of various dehydrogenase enzymes found in mycorrhizal fungi, particularly SDH (succinate dehydrogenase). NBT is a yellow tetrazolium salt, which is reduced to purple formazan. Other tetrazolium salts have also been used in various investigations. FDA was used to determine the activity of mycorrhizal fungi. FDA passively enters the cells down a concentration gradient and is hydrolysed by esterases to release free fluorescin, which fluoresces at a wavelength of 490 nm. The usefulness of these vital stains differs depending on the situation: NBT is particularly useful in staining root segments or sections and distinguishes fungal structures, the phloem in the vascular system, and passage cells in the hypodermis. Thick (120 μm) sections of the roots can be cut using a freezing microtome. This slicing does not appear to kill the fungus, which has the ability to reseal the cut ends. Cut cells of the root are killed and do not take up the stain, thus allowing the fungal cells to be differentiated from the plant cells. FDA is unsuitable for use on root sections because of autofluorescence in both plant and fungal walls, which makes it difficult to demarcate the area occupied by an active fungus. FDA is useful in staining external hyphae. A soil sample is diluted with distilled water, which is stirred rather than blended to avoid possible damage to hyphae. Aliquotes of this solution are taken using a Millipore filter set-up which produces a thin layer of residue on the filter. Purple staining with NBT gave poor contrast between the hyphae and the dark background of soil particles; FDA worked better and produced a strong fluorescence that contrasted clearly with the background (Sukarno and Dickson 1992).

In studies conducted at the Department of Land Resource Science, University of Guelph, Ontario, Canada, a modified vital staining technique, used in intraradical VAM colonization, was applied to extraradical VAM hyphae in order to understand how

VAM fungal mycelia respond to environmental stresses such as freezing and disturbances such as tillage. In this method, hyphae are extracted from soil, collected on nylon mesh filters, and incubated in NBT succinate solution (NBT succinate). Succinate dehydrogenase, which is present in viable fungal hyphae, reacts with NBT and is readily reduced to a dark blue or purple formazan compound. The hyphae are then fixed, cleared in KOH, and counterstained with AF (acid fuchsin). This counterstaining not only increases the contrast between viable and non-viable hyphal segments but also allows total hyphal length to be measured from the same slide used for measuring hyphal viability. Both formazan and AF will persist for extended periods if samples are stored in the dark, whereas fluorescent stains fade quickly, which limits their use to immediate observations of samples. This vital staining technique also has the advantage of being relatively inexpensive and simple to use. However, the stains do not distinguish between mycorrhizal and non-mycorrhizal fungi. Such differentiation can be obtained on the basis of morphological characteristics (Heather, Shaffer, and Miller 1993).

In further studies conducted at the Department of Botany of the University of Guelph, the NBT succinate method was further modified for the assessment of SDH activity in leek, maize, and pigmented soybean colonized by VAM fungi. Roots treated with NBT succinate, cleared in (w/v) KOH in a drying oven (55 °C) for 24 hours or longer, and observed as whole mounts revealed signs of intraradical VAM fungus colonization more clearly than the roots cleared by the standard method of boiling in 20% (w/v) chloral hydrate. A combination of the two techniques by clearing roots in boiling chloral hydrate followed by clearing in 5 KOH for prolonged periods also improved the visibility of intraradical fungal structures. Bleaching the roots treated with NBT succinate using the standard NH_3-H_2O_2 method removed pigmentation from roots and did not affect the viability indicator, namely formazan. Roots of pigmented soybean collected from the field were successfully cleared and bleached to reveal the signs of viable and non-viable intraradical fungal structures. Counterstaining the roots with AF made both viable and non-viable intraradical fungal structures clearly visible. The NBT-succinate solution infiltrated all intraradical fungal structures after 24 hours. Formazan products were observed at similar concentrations in viable structures after 24, 36, and 48 hours (Schaffer and Peterson 1993).

Extraction of hyphae from VAM roots

In studies conducted at the National Grassland Research Institute, Nishinasuno, Tochigi, Japan, on isolation of metabolically active internal hyphae from arbuscular mycorrhiza in onion, a method was described wherein roots of onion, colonized by *Gigaspora margarita* were digested for one hour at 30 °C with solution containing cellulase and pectinase and then homogenized with a blender at a low speed. The internal hyphae were collected from the homogenate by centrifugation on a discontinuous gradient of Percoll and then purified further by filtration. Enzyme histochemical staining showed that the collected internal hyphae had active SDH and 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 extracted from axenically germinated spores of *Gi. margarita*. The specific activities of hexokinase and of the enzymes involved in phosphate metabolism alkalinease acid and phosphatases and in the TCA cycle (SDH and 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 Department of Agricultural Biochemistry, Waite Agricultural Research Institute, Glen Osmond, Australia, enzymic activity of hyphae and vesicles of the VAM fungus *Glomus intraradices* in the roots of *Allium porrum* was measured before and after enzymic digestion of the fungus. Using NBT to detect the activity of SDH and the fluorescence of acridin orange, the researchers observed a decline in the activity of enzymes of the mycorrhizal fungus during enzymic digestion. No enzymic treatment was found that permitted removal of the fungus from roots without plasmolysis of vesicles and a reduction in enzyme activity (McGee and Smith 1990).

Quantification of active VAM infection

Most research on the interactions between mycorrhizal fungi and plants requires the determination of the extent to which the fungi colonize the root systems. It is, however, important to obtain data that give a better estimate of the intensity (in terms of root volume or mass infected) and the quality of infection (for example, arbuscules or vesicle production) and the proportion of propagules that remain viable over different duration (Smith and Dickson 1990).

In another study by the same researchers, image analysis was used to quantify different parameters of infective propagules which include numbers of cells containing arbuscules relative to the total number of infected cells in the root cortex, the proportion of the root volume colonized by arbuscular infection, and the activity of arbuscules as shown by the intensity of staining of SDH with NBT. The results indicated that over a period of nine weeks, the number of cortical cells containing arbuscules and the activity of SDH in individual arbuscules declined appreciably (Smith and Dickson 1990).

Yet another study by the same team describes the methods for objective measurement of VAM infection in the roots of *Trifolium subterraneum*, peas, leeks, and cucumber based on vital staining of transverse sections of fresh roots, followed by evaluation of the infection and determination of the number of living arbuscules and hyphae in each transverse section. The most useful method involved freeze-sectioning of roots embeded in gelatin containing glycerol and DMSO, followed by staining the sections with NBT overnight to reveal SDH activity. Two systems of computer-aided image analysis were evaluated for the determination of the number, perimeter, and cross-sectional area of the intercellular hyphae and arbuscules. This approach, which is clearly objective, is an advance over the earlier methods based on ranking sections or segments of root based on the intensity of development of arbuscules. The data were used to calculate the area of interface between the plant and the fungus in infected roots. The results showed the contribution of arbuscules to the total infection. Also, the area of interface declined markedly with age of the plants and the infection (Smith and Dickson 1991).

In further studies, Smith and Dickson (1992) refined the method of detection of active VAM infection by vital enzyme staining. The fungus was quantified by observing transverse sections of roots using the chromatic colour image analysis system (L R Jarvis, Leading Edge). Automatic discrimination was achieved by selecting the average colour intensity of the fungal structures within the sections. This segment of the image is then digitized and used for calculating fungal areas, perimeters, and the numbers of different structures including hyphae, vesicles, and arbuscules. For soil samples, the length of active hyphae was determined by using a grid intersect placed in the eyepiece of a fluorescence microscope.

In studies conducted in the Soil Microbiology Department, Estacion Experimental del Zaidin, Granada, Spain, the percentage of infected root length and SDH activity were assessed in maize, cucumber, and wheat inoculated with *Glomus mosseae*. The results indicated that although the histochemical assay of SDH detects the metabolic function of the fungus, quantifying this activity as a percentage of mycorrhizal root length along which the activity was observed was not indicative of the efficiency of the fungus in promoting plant growth (Vierheilig and Ocampo 1990).

Use of enzyme staining in studying the dynamics of VAM infection

Vital staining methods have been used in tracking the course of VAM infection in time and space and to study the effects of fungicides, phosphates, environmental factors, and interactions with other microorganisms in VAM infections.

The course of VAM infection

In studies conducted at the Instituto Coltivazioni Arboree dell' Universita, Via Giuria 15, Turin, Italy, activities of acid and alkaline phosphatase, esterase, and succinate dehydrogenase were assessed in extraradical mycelium of *Glomus clarum* by histochemical methods. Staining was observed for all enzymes except acid phosphatase. The amount of active mycelium decreased after the initial infection. In older hyphae, stained deposits were irregularly distributed inside the hyphae. It is possible that in some hyphal tracts the enzymatic activity was reduced following the formation of vacuoles (Schubert and Mazitelli 1990).

In studies conducted in the Plant Science Department of Macdonald College, McGill University, Ste-Anne de Bellevue, Quebec, Canada, the extraradical hyphae of *G. intraradices* associated with a mixture of Saranac lucerne and bromegrass (*Bromus inermis* cv Tempo) were tested for metabolic activity by three enzyme-staining procedures. Mycorrhizal plants were grown for 6, 9, and 12 weeks and the vital staining methods were evaluated and compared. The SDH activity was assessed by the reduction of NBT and NADH diaphorase activity by the reduction of INT (indonitro tetrazolium). The FDA (fluorescin diacetate) hydrolysis was used to assess the activity of esterases in the extraradical hyphae. Percentage of intraradical hyphae active in lucerne and bromegrass were determined after staining root samples with NBT and chlorozol black E. The percentage of live intraradical infection declined as the symbiosis matured, whereas that of extraradical hyphae did not (the NBT, however, did not show this contrast) suggesting a different turnover for extra- and intraradical hyphae. The FDA gave the best estimates of enzyme activity, followed by INT and NBT in that order (Hamel, Fyles, and Smith 1990).

In studies conducted in INRA, CNRS, Phytoparasitol laboratory, Genet & Ameliorat Plantes, Dijon, Cedex, France, a histochemical procedure was developed to estimate the proportion of AM infections showing fungal alkaline phosphatase activity in the total amount of fungal tissue (indicated by trypan blue staining) and in the living mycelium (indicated by SDH activity). In roots of leek and *Platanus acerifolia*, only a small proportion of living intraradical mycelium showed alkaline phosphatase activity during early infection by *Glomus* spp. but this increased greatly just before the mycorrhizal growth response of the host plant. Infection revealed by all three stains reached a maximum at six weeks after inoculation after which the level of trypan-blue-stained infection remained constant whereas that showing SDH and alkaline phosphatase activity declined. Alkaline phosphatase activity was absent from virtually all abortive entry-point hyphae formed on the roots of a resistant myc (–), nod (–) mutant of pea, although SDH activity

was detected. The results indicate that alkaline phosphatase activity is induced by colonization of host roots and that this fungal enzyme could be a useful marker for analysing the symbiotic efficiency of AM infection (Tisserant, Gianinazzi-Pearson, Gianinazzi, *et al.* 1993).

Further studies were conducted in the same laboratory to define the relationships between root order dynamics, AM development, and the physiological state of symbiotic *Glomus fasciculatum* in a woody plant species, *Platanus acerifolia*, using SDH and alkaline phosphatase to detect active mycelium. Arbuscular mycorrhiza influenced root system development in *P. acerifolia* and was closely related to the appearance of different root orders. The most active mycelium, characterized by fungal SDH and alkaline phosphatase activities, was mainly localized in the newly formed lateral roots. Nine weeks after inoculation with *G. fasciculatum*, the proportion of mycelium showing the activity of alkaline phosphatase strongly decreased in all root orders, probably because of increased phosphorus content of the host plant (Tisserant, Gianinazzi, and Gianinazzi-Pearson 1996).

In studies conducted at the Department of Plant Pathology, Kansas State University, Manhattan, Kansas, USA, the effect of burning on the development of mycorrhizal symbiosis as determined by enzyme staining was determined in big bluestem *Andropogon gerardii*. Burning is an integral part of tall grass prairie maintenance and regularly stimulates plant growth and productivity. Eight days after burning, no differences were found in per cent root colonization between burned and unburned plots as determined by staining with trypan blue. However, in SDH assays, burning increased the percentage of root tissues colonized by metabolically active mycorrhizal fungi. This increase in mycorrhizal activity may contribute to the burst of plant growth following burning (Bentivenga and Hetrick 1990).

In studies conducted at the Soil Science Department, AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts, UK, the SDH activity in hyphae of the VAM fungus *G. mosseae* in symbiotic association with leek (*Allium porrum*) was investigated by histochemical staining in situ. Leek seedlings were transplanted to sand culture and inoculated with spores of *G. mosseae* placed just below the base of the stem. At 14, 25, 35, and 60 days after transplanting, the growth medium (sand) was flooded with NBT chloride solution, thereby displacing the nutrient solution. This allowed the sites of SDH activity in external and internal fungal structures to be stained without physically disturbing the symbiotic system. After counterstaining the harvested roots and mycelium with AF, it was possible to differentiate clearly between the metabolically active and inactive regions of the fungus. The lengths of both external hyphae and infected roots increased exponentially and were in constant proportion (1.4 m hyphae per cm of infected root) for up to 60 days. The percentage length of external hyphae with SDH activity remained almost constant (80%). In each infected length of root, there was a gradation of SDH activity from the inactive distal (older) hyphae to uniformly active proximal (younger) hyphae (Saito, Stribley, and Hepper 1993).

Studies were conducted at the Department of Botany, Mansoura University, Mansoura, Egypt, to investigate the response of soybean (*Glycine max*) to inoculation with VAM fungus *G. mosseae* at flowering and maturity stages of plant growth. Activity of fungal SDH and alkaline phosphatase indicated that the response of soybean to mycorrhizal infection was higher at flowering at maturity. The level of total mycorrhizal infection was not generally related to plant growth and phosphorus content (AbdelFattah 1997).

Studies conducted in the College of Life Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, China, compared two VAM fungi, *G. mosseae* and *G. intraradices*, for abundance of intraradical and soil-borne hyphae in association with *Astragalus sinicum*, a legume with small seeds, and *Glycine max* by enzyme staining. *A. sinicum* was more responsive than *G. max* to mycorrhizal formation, especially in early growth stages. Biomass allocation was greater in roots than shoots for mycorrhizal *A. sinicum* while the opposite was true for *G. max*. Hyphal development in root and soil compartments was estimated by trypan blue staining and SDH and alkaline phosphatase activity. Total fungal abundance increased steadily in roots and soil with time, leading a maximum eight weeks after sowing. Activity of SDH and alkaline phosphatase in AM hyphae increased in roots during plant growth but decreased in soil at later harvests. Mycorrhizal root mass in *A. sinicum* and *G. max* increased about 14-fold and 2.5-fold, respectively, but total length of soil hyphae produced per plant differed little so that the pattern of abundance of the two fungi in soil and in roots varied considerably with the host plant (Zhao, Trouvelot, Gianinazzi, *et al.* 1997).

In studies conducted at the University of Pisa, CNR, Centre Studio Microbiol Suolo, Departmento and Biotecnol Agrarie, Via Borghetto 80, Pisa, Italy, enzyme staining was used to find out protoplasmic continuity in hyphal fusions of VAM fungi. Studies conducted on *G. mosseae* colonizing roots of *T. vulgaris* and *P. cerasifera* showed that the mycelium spreading from the colonized roots of *T. vulgaris* and *P. cerasifera* showed hyphal fusions to the extent of 64% and 78%, respectively, of the total contacts occurring between the hyphae amounting to 0.66 and 0.51 anastmoses per mm of the hyphae, respectively. Histochemical localization of the SDH activity in hyphal bridges demonstrated protoplasmic continuity, while the detection of nuclei in the hyphal bridges confirmed the viability

of anastomosed hyphae. The ability of extraradical mycelium to form anastomoses and to exchange nuclei suggests that beyond nutritional flow, flow of information might also be active in the network (Giovannetti, Fortuna, Citernesi, *et al.* 2001).

In studies conducted at the Department of Agricultural Biochemistry, Waite Agricultural Research Institute, on the effect of photon irradiance on phosphorus uptake and growth of onion inoculated with *G. mosseae*, NBT chloride was used as a vital stain for SDH activity. During the 6-week-long experiment, all the infections revealed by trypan blue staining were physiologically active. Branched arbuscules always had SDH and alkaline phosphatase activity. Thus, in young plants, observing of arbuscules gives a good indication of the amount of active infection (Smith and Gianinazzi-Pearson 1990).

Effect of phosphorus on metabolic activity of VAM as determined by enzyme staining

In studies conducted at the Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata-Ken, Japan, on the effect of phosphate application on the matabolic activity of hyphae in VAM root, onion seedlings were transplanted in 200-ml pots filled with autoclaved sand and inoculated with *Gi. margarita*. The seedlings were grown in growth chambers and phosphate-free nutrient solution was applied every two days. (At 40 days after transplanting, three treatments were given: injection of 0.1% P solution to shoots, (2) application of 8 mg P/L nutrient solution, and (3) application of P-free nutrient solution). As before, the solutions were applied every two days. At 40 and 80 days after the treatment, metabolic activity of the internal hyphae was evaluated with SDH. After the enzymatic staining, the hyphae were counterstained with AF. The percentage of root length infected, number of appressoria per unit root length, and shoot weight were measured. Shoot growth was increased a little in P-free treatment. VAM infection was decreased in plants that received P. The number of appressoria per unit length of infected root decreased with time and was not affected by the treatments. There was a significant positive correlation between the number of appressoria per plant and the length of infected roots per plant. At 40 days after treatment, the percentage of hyphae showing SDH activity did not differ among the treatments. The results suggest that metabolic activity of internal hyphae is little affected by phosphate application for a short period and that the inhibition of infection by phosphate application occurs at the stage of hyphal penetration of roots. (Tawaraya, Saito, Morioka, *et al.* 1992 and 1994)

Studies conducted at the Laboratore de Phytoparasitology, INRA-CNRA, Station de Genetique et d' Amelioration des Plantes, Dijon, Cedex France, on the effects of phosphate application fertilization on the physiological activity of VAM infection in soybean and pineapple used fungal SDH and alkaline phosphatase. Soybean and pineapple were used because of their different growth rates and response to phosphate. Total mycorrhizal infection as estimated by trypan blue staining was found to be reduced by phosphate application soils with adequate levels of P. In P-deficient soils, fungal infection in pineapple roots was not affected by fertilizer application. The level of total mycorrhizal infection was not related to plant growth. Fungal alkaline phosphatase staining and to a lesser extent, SDH activity were related to plant growth. This procedure is thus potentially useful in estimating endomycorrhizal infection as a measure of efficiency of the symbiosis (Guillemen, Orozco, Gianinazzi-Pearson, *et al.* 1995).

Effect of fungicides on metabolic activity of VAM as determined by enzyme staining

Studies conducted at the Station d' Amelioration des Plantes, INRA, Dijon, Cedex, France, showed that application of either benomyl or captan to soil significantly decreased the growth VAM-inoculated onion four weeks after the treatment but not that of non-VAM plants. Fungal colonization of onion as indicated by non-vital staining with chlorozol Black E was depressed two weeks after fungicide application. However decrease in metabolically active VAM fungal tissue revealed by SDH assay could be detected as early as three days after the treatment. There was little difference between the fungicides in their effect on VAM fungi used. SDH assay is thus useful in studying the effects of fungicides (Kough, Gianinazzi-Pearson, and Gianinazzi 1987).

Studies conducted at the Department of Plant Pathology, J N Agricultural University, Indore Campus, India, on the effect of fosetyl Al (tris-o-ethyl phosphorate), a systemic anti-oomycetes, symplast fungicide, used at three concentrations for seed and foliar application on colonization of soybean roots by *G. mosseae* and *G. fasciculatum* showed that the fungicide treatment increased colonization, intramatrical vesicles, arbuscules, and extramatrical spores significantly. SDH assay showed greater colonization and increased growth and yield of soybean in plants treated with the fungicide (Vyas 1990).

In experiments conducted at the Department of Microbiology, Swedish University of Agricultural Sciences Uppsala, Sweden, the influence of two systemic fungicides, propiconazol, and carbendazim (bavistin), at field application rates on the functions of three VAM fungi was studied. Short-terms ^{32} P-transport and SDH activity in external hyphae of *G. intraradices*, *G. claroideum*, and *G. invermanium* in symbiosis with pea (*Pisum sativum*) were measured. In the experimental system, the hyphae grew into two root-free hyphal compartments. The fungicides were applied to both

compartments 24 days after sowing and ^{32}P was added to only one in each pot. Four days later, the effect of the fungicide on fungal P transport was measured as the difference in ^{32} P content in treated and untreated plants. SDH activity in fungal hyphae was determined in hyphal compartments with no ^{32} P. Carbendazim severely inhibited ^{32} P transport and SDH activity in external hyphae at an application rate of 0.5 µg per litre of soil. The ergosterol inhibitor, propiconazol, affected none of these parameters. The fungicides had similar effects on all the three fungal species although P-transport efficiency and SDH activity differed markedly among the species (Kling and Jakobsen 1997).

Effect of interactions with other soil microorganisms on metabolic activity of VAM as determined by enzyme staining

Studies conducted at CSIS, Estacion, Experimental del, Zaidin, Department of Microbiology, Suelo and Sistemas Simbiont, Granada, Spain, compared the effect of two *Rhizobium mililoti* strains, the wild type (WT) and its GM (genetically modified) derivate, on physiological activity of the VAM fungus, *G. mosseae*, during the colonization of alfalfa (*Medicago sativa*) roots as affected by the water status in the growing medium. Activities of SDH and alkaline phosphatase were used as a measure of the effect. The VAM plants did not differ significantly in their growth response to the two strains at any water level. This lack of response did not adversely affect the development of AM symbiosis. In well-watered plants, SDH activity indicated that about 80% of the AM mycelium in plants inoculated with GM Rhizobium were alive throughout the experiment compared to only 10%–20% in those inoculated with the WT strain. Both the rhizobial strains behaved similarly under water-limiting conditions in regard to AM development (Vazquez, Bejarano, Azcon, *et al.* 2000).

In another study at the same department, SDH was also used to study the effect of two soil fungi, *Alternaria alternata* and *Fusarium equiseti*, on metabolic activity of extraradical hyphae of *G. mosseae*. Both the saprophytes decreased plant dry weight and mycorrhization when introduced into the rhizosphere before inoculation with *G. mosseae* but they did not affect the percentage of root length colonized by the AM endophyte when inoculated two weeks after *G. mosseae*. However, these saprophytes did affect the metabolic activity of *G. mosseae* as assessed by the SDH activity. The results suggest that the saprophytic fungi may affect the mycorrhizal fungi directly in the extramatrical phase of the latter but less so after the AM fungus is established affected less (McAlister, Garcia Garrido, *et al.* 1997).

Studies conducted at the Department of Ciencies Biologicas, Universidad de Buenos Aires, 1428 Buenos Aires, Argentina, on the interaction between *Aspergillus niger* and *G. mosseae* showed that percentage germination and the length of hyphae of germinated spores of *G. mosseae* decreased significantly in the presence of *A. niger* on both water agar and malt extract agar. Shoot dry weight and percentage of AM colonization of roots of maize and lettuce decreased when *A. niger* was inoculated at the same time or two weeks before *G. mosseae* but unaffected when *A. niger* was inoculated two weeks after *G. mosseae*. However, metabolic activity resulting from AM colonization, measured as a percentage of mycelium showing SDH activity decreased in all the treatments (McAllister, Garcia-Romera, Martin, *et al.* 1995).

Further studies conducted at the CSIC, Department Microbiology, Estacion Experimentala Zaidan, Granada, Spain, on the interaction between *G. mosseae* and *Trichoderma koningii* or *Fusarium solani* showed that *T. koningii* decreased plant dry weight and AM colonization on roots of maize and lettuce when inoculated into the rhizosphere before or at the same time as *G. mosseae* but had no effect when inoculated two weeks later. The effect of *F. solani* on the AM colonization of lettuce roots was similar to that of *T. Koningii* although *F. solani* did not affect AM colonization of maize roots. *T. koningii* adversely affected the metabolic activity of AM mycelium as assessed by SDH activity in all the treatments (McAllister, Garcia-Romera, Godeas, *et al.* 1994).

Studies conducted at the University Nacional Agraria la Selva, Faculty Agronomy, Tingo Maria, Huanaco, Peru, on single or dual inoculation of alfalfa (*Medicago sativa* cv. Aragon) with *Rhizobium meliloti* strain 10 F28 and *G. fasciculatum* showed that dual-inocualted plants had higher shoot dry weight, the lowest root-to-shoot ratio, and higher oxygen uptake and nodule nitrogenase activity than those inoculated with either of the two organisms. However, dry weight of the roots of plants inoculated with the VAM alone was higher than those from other treatments. These differences were not correlated with SDH activity, which was at a similar level in all the treatments. The data suggest that *Rhizobium* may affect metabolism and that the effect is not achieved via the tricarboxylic acid pathway (Piccini, Ocampo, and Bedmar 1988).

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Research findings

Effect of dual inoculation (AM fungi and *Rhizobium*) on chlorophyll content of *Vigna unguiculata* (L). Walp. var. Pusa 151

S Rajasekaran and *S M Nagarajan*
Department of Botany, A V C College, Mannampandal – 609 305, India

The symbiotic relationship between VAM (vesicular arbuscular mycorrhiza) fungi and the host plants has been studied traditionally in terms of benefits to the individual plant and fungi (Smith and Smith 1996; Francis and Read 1995). VAM association can affect the host plants in terms of stomatal movement and photosynthesis of leaves and has been shown to increase chlorophyll content and the rate of transpiration and photosynthesis (Panwar 1991; Bethlenfalvay, Brown, and Franson 1988). Mycorrhizal infection is of particular value to legumes because it can increase the P (phosphorus) update: nodulation and symbiotic nitrogen fixation by rhizobia require adequate supply of P, and restricted root system leads to poor competition for soil P (Carling, Richle, and Johnson 1978).

Cowpea – *Vigna unguiculata* (L). Walp – is one of the principle pulses of India, grown as a vegetable in most parts of the country. Fully mature seeds of cowpea are boiled and eaten; they are also split and used as dal. The seed contains 24% protein and is a rich source of calcium and iron (CSIR 1967). In light of these facts, the present investigation was undertaken to study the effect of dual inoculation (VAM fungi and *Rhizobium*) on the chlorophyll content of *V. unguiculata* leaves.

Material and methods

Seeds of *V. unguiculata* (L.) Walp. Var. Pusa 151 were obtained from the Agricultural Research Station, Vamban, Pudukkottai district, Tamil Nadu. Seeds free from visible defects were selected for the study. Seedlings were raised in pots containing a mixture of sand and soil in equal proportion. Seeds were inoculated either with *Rhizobium* or with *Glomus mosseae* (applied as a layer on soil surface) or with both together. One pot of seedlings was left without inoculation as control. The leaves were collected for analyses after 15, 25, and 40 days of inoculation and the chlorophyll content was estimated using the method of Arnon (1949). All the experiments were carried out in triplicate and the results were expressed as the average of the three replications.

Results and discussion

Plants inoculated with VAM fungus alone or in combination with *Rhizobium* showed significant changes in chlorophyll a and b and total chlorophyll content. The total chlorophyll content was the highest in dual-inoculated plants, particularly in five-day-old leaves (see table below). Such an increase might be due to an increase in the rate of photosynthesis and transpiration or increased growth (Sampathkumar and Ganeshkumar 2003; Hayman 1983) or due to the presence of a large number of chloroplasts in the bundle sheath of inoculated leaves (Krishna and Bhagyaraj 1984). Several studies reported indicate that chlorophyll content is higher in the leaves of resistant varieties than those of the susceptible ones as biochemical characters like phenols, proteins, and chlorophyll may play a vital role in making plants resistant to pathogens (Bhavani, Venkatasubbaiah , Sudhakar Rao, *et al.* 1998; Charitha Devi and Reddy 2001). Thus the present study clearly reveals that there is an increase in the total chlorophyll content, because the association of VAM, *Rhizobium*, and the host is believed to have a possible effect on host defence mechanism and photosynthesis.

It is concluded that dual inoculation with mycorrhizal fungi and rhizobium species is effective in increasing the chlorophyll content, leading to enhanced growth in legumes.

Effect of arbuscular mycorrhiza fungi and *Rhizobium* on chlorophyll content of cowpea

Treatment	*Chlorophyll A* (mg/g)				*Chlorophyll B* (mg/g)				*Total chlorophyll* (mg/g)			
	Days after inoculation			*Mean*	*Days after inoculation*			*Mean*	*Days after inoculation*			*Mean*
	15	25	40		15	25	40		15	25	40	
Control	0.61	0.73	0.82	0.72	1.04	1.12	1.18	1.11	2.06	2.15	2.24	2.15
Rhizobium	0.71	0.76	0.97	0.79	1.10	1.21	1.32	1.21	2.14	2.31	2.43	2.29
Glomus mosseae	0.63	0.81	0.88	0.77	1.12	1.26	1.21	1.16	2.16	2.35	2.28	2.26
Rhizobium and *G. mosseae*	0.77	0.92	1.07	0.92	1.22	1.25	1.64	1.37	2.27	2.35	2.73	2.45
Mean	0.68	0.80	0.93	–	1.12	1.21	1.33	–	2.15	2.27	2.42	–

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Germination response of mango stones to different arbuscular mycorrhiza fungi

Santosh C P Patil and *P B Patil*
Kittur Rani Channamma College of Horticulture, Arabhavi – 591 310, India

Because mango stones being recalcitrant, rapidly lose viability (Ruehle and Ledin 1955), they should be collected and sown within a week of collection, especially in the high-latitude tropics and arid and semi-arid regions where high evaporative demand prevails during the period when stones are available. In these areas, mango is being propagated on nondescript monoembryonic rootstocks following ex situ propagation (container-grown, detached grafting) with 30% to 40% germination. Good and early germination of mango stones (seeds) is the main prerequisite to successful graftins.

Though Ram (1997) achieved good germination by soaking the stones in or spraying them with gibberellins, the seedlings were unsatisfactory. According to Reddy (1991), mango responds well to the AM fungal inoculation, in the form of increased girth, height, and leaf area. Earlier workers have reported that the AM fungi secrete plant growth regulators like gibberellins (Miller 1971; Crafts and Millar 1974; Slankis 1975), which are known to promote seed germination in mango and other crops. In order to use these benefits and to find out the most efficient AM fungi for germination and growth in mango, the present experiment was undertaken.

Materials and methods

An investigation on the effect of the AM fungi on germination in mango stone was carried out in the Department of Pomology, KRC College of Horticulture, Arabhavi, during 2003/04. The

experiment was laid out in completely randomized block design with ten treatments (nine AM fungi and one uninoculated control) and four replications. Two hundred seed stones were sown per treatment per replication in 18 x 12 cm polybags filled with potting mixture (soil, sand, and FYM in the ratio of 2:1:2). Inoculation with the AM fungal was carried out by placing 5 g of inoculum uniformly, which consisted on an average of 19 chlamydospores per gram at a depth of 5 cm. After putting a thin layer of soil on the inoculum, stones from Alphonso mangoes obtained from a single lot of uniform size and shape from a processing unit were placed and covered with soil. Days required for emergence of the plumule (germination), days taken for 50% germination, days taken for completion of germination, germination percentage, germination vigour index, the number of chlamydospores, and per cent root colonization were recorded.

The GVI (germination index) was computed using the formula

$$GVI = \frac{X_1}{D_1} + \frac{X_2}{D_2} + \frac{X_3}{D_3} + \ldots\ldots\ldots\ldots + \frac{X_n}{D_n}$$

where, $X_1, X_2, X_3 \ldots X_n$ were the number of seeds germinated and $D_1, D_2, D_3 \ldots D_n$ were the days taken for germination.

The number of extrametrical chlamydospores produced by the AM fungi were determined by using wet sieving and decanting method as described by Gerdemann and Nicolson (1963). The per cent root colonization was determined using the method as described by Phillips and Hayman (1970). The data recorded on various parameters were subjected to Fisher's method of variance and interpretation of data was done as described by Panse and Sukhatme (1967).

Results and discussion

Days required for emergence of plumule

Mango stones inoculated with *Glomus leptotichum, Acaulospora laevis, Glomus intraradices*, and *Gigaspora margarita* required the least number of days (7.00) for initiation of germination (emergence of plumule). However, these values were statistically at par with the days taken for germination of seeds inoculated with *Glomus fasciculatum* and *Glomus monosporum* (7.50 days) and control (8.00 days). Inoculation with *Glomus bagyaraji* (13.00 days), *Sclerocystis dussii* (10.75 days), and *Glomus mosseae* (10.75 days) led to significantly more days for initiation of germination than those recorded with control (8.00 days) or with other treatments (see table below).

Days taken for 50% germination

Whereas the days taken by those mango stones for 50% germination, inoculated with *G. leptotichum, A. laevis*, *G. intraradices*, *G. fasciculatum*, *G. monosporum,* and *Gi. margarita* (17.00) were statistically on par, (15-17 days). Control seedlings attained 50% germination within 20 days of but those inoculated with *G. mosseae* (24.00 days), *S. dussii* (23.00 days), and *G. bagyaraji* took much longer (22 days).

Days taken for completion of germination

The data (see table below) indicate that the mango stones inoculated with *Gi. margarita* and *A. laevis* took significantly fewer days to complete germination (100%) whereas those inoculated with *G. leptotichum*, *G. intraradices* , *G. mosseae*, *G. fasciculatum*, *G. monosporum,* and *S. dussii* took longer, and those not inoculated at all (control) or with *G. bagyaraji* took the longest time.

Effect of different arbuscular mycorrhiza fungi on germination of mango stones and microbial parameters

Treatment	*Number of days taken for germination*			*Germination (%)*	*Germination*	*Spore count index*	*Root colonization (%)*
	Initiation	*50%*	*Completion*				
T_1 – *Sclerocystis dussii*	10.75	23.00	44.75	28.76	52.89	399.00	86.75
T_2 – *Glomus leptotichum*	7.00	15.00	44.00	57.25	169.76	412.00	86.50
T_3 – *Acaulospora laevis*	7.00	17.00	42.00	65.00	192.91	657.00	92.75
T_4 – *Glomus intraradices*	7.00	17.00	44.00	55.93	179.36	411.75	86.00
T_5 – *Glomus bagyaraji*	13.00	22.00	46.00	28.00	52.86	387.50	84.25
T_6 – *Glomus mosseae*	10.75	24.00	44.75	46.35	78.48	423.75	85.75
T_7 – *Glomus fasciculatum*	7.50	17.00	44.75	58.43	179.17	656.00	93.00
T_8 – *Glomus monosporum*	7.50	17.00	44.75	64.06	201.79	576.75	88.75
T_9 – *Gigaspora margarita*	7.00	17.00	42.00	65.00	205.36	669.75	93.00
T_{10} – Control	8.00	20.00	47.25	49.72	160.90	60.50	20.00
S. Em ±	0.77	1.01	0.855	0.73	1.13	50.65	1.93
C.D. at 5%	2.29	2.92	2.47	2.09	3.25	146.27	5.59

C.D. – critical difference; S.Em – standard error of mean

Germination percentage

Inoculation of mango stones with *A. laevis* and *Gi. margarita* resulted in significantly maximum germination percentage (65.00%) followed by inoculation with *G. monosporum* (64.06%) and *G. fasciculatum* (58.43%). These treatments were significantly superior over control and rest of the treatments. Significantly minimum germination percentage was recorded in stones inoculated with *G. bagyaraji* (28.00%), *S. dussii* (28.76%), and *G. mosseae* (46.35%) when compared to control (49.72%) and other treatments.

Germination vigour index

Germination index ranged from 52.86 to 205.36. *Gi. margarita* had shown significantly highest germination index (205.36) followed by *G. monosporum* (201.79) and *A. laevis* (192.91), which were significantly higher than control (160.90). Significantly lower values of germination index were recorded in the treatments with *S. dussii* (52.89) and *G. bagyaraji* (52.86) followed by *G. mosseae* (78.48), even much lower than the germination index values obtained in control.

Chlamydospores

Significantly maximum extramatrical chlamydospores were present in the germinating media inoculated with *Gi. margarita* (669.75/50 g of soil), which were statistically similar with the counts in the media inoculated with *A. laevis* (657.00/50 g of soil), *G. fasciculatum* (656.00/50 g of soil), and *G. monosporum* (576.75/50 g of soil). Significantly lower numbers of chlamydospores were recorded in control soils (60.50/50 g of soil). Least numbers of chlamydospores were recorded in the potting mixture inoculated with *G. bagyaraji* (387.50/50 g of soil) and *S. dussii* (399/50% g of soil).

Per cent root colonization

Root colonization by the AM fungi ranged from 84.25% to 93%. Stones inoculated with *Gi. margarita* and *G. fasciculatum* (93.00%) recorded significantly higher root colonization (mycorrhization), which was statistically at par with *A. laevis* (92.75%) and *G. monosporum* (88.75%). The uninoculated stones showed significantly least root infection (20.00%).

The stones inoculated with *Gi. margarita, A. laevis, G. monosporum*, and *G. fasciculatum* recorded higher percentage of germination, with higher germination index of 205.36 to 169.76 which was attained in a span of 42 to 44.75 days, recording significantly in much lower time span when compared to the germination in control stones.

Although there are no reports available to explain the role of AM fungi in initiation of early germination of mango stones, the increased germination, germination index, and fewer days taken for germination observed in the present investigation can be attributed to a number of sequential events that occur, beginning with the presence of chlamydospores in the rhizosphere (germinating medium) of mango stones. The initiation of AM association begins with the germination of the spore and the contact of fungal hyphae with root epidermis. There is a molecular and chemical dialogue that occurs between the AM fungi and host (Giovannetti, Avio, Sbrana, *et al.* 1993; Harborne and Williams 2000). Plant exudates from the appropriate host are necessary to initiate the symbiosis. The leachates from mango stones might have influenced the germination of chlamydospores and hyphal growth. Further, spore germination and some independent hyphal growth of the AM fungi can occur in absence of roots and this saprophytic phase is crucial to the fungal life cycle as survival depends on the ability to rapidly and effectively infect suitable hosts. Thus, the AM fungi germinate and some independent hyphal growth is achieved until the emergence of the radical, which emerges much sooner than the plumule does. Probably the radical gets infected as soon as it emerges. Further, certain of the introduced AM fungi, being efficient root colonizers (Table 1), might establish well in the rhizosphere (Manjunath, Mottan, and Bagyaraj 1983). High level of root infection by the AM fungi might affect further growth, namely the growth of roots and shoots (Shivashankar and Iyer 1988).

Mycorrhizal fungi are known to produce Auxins, cytokinins, gibberellins, and vitamins in pure culture (Miller 1971; Crafts and Millar 1974; Slankis 1975), and the effects of these compounds on plant growth and development are well documented (Torrey 1976). Gibberellins have been found to be effective in increasing mango germination (Ram 1997). The increase in germination the percentage with higher germination index and faster germination observed with a few AM fungi in the present investigation could be attributed to the production of gibberellic acid by the AM fungi. Gange, Brown, and Sinclair (1993) experimentally demonstrated that the AM fungal presence of the AM fungi affects seed germination of a secondary succession site.

Results of the present investigation using nine AM fungi on mango stone germination did not reveal any uniform response, which indicates that the extent of the AM fungal association with the host is also a morphogenetically determined phenomenon. In spite of the fact that the AM fungi are not host-specific (Smith and Read 1997), they vary widely in their effectiveness. It was surprising to note that inoculation with *Gi. margarita, A. laevis, G. monosporum*, and *G. fasciculatum* significantly increased the germination whereas that with *G. bagyaraji, S. dussii,* and *G. mosseae* decreased it, compared to the control. Despite the lack of absolute

specificity accorded to the AM symbiosis, the symbiotic efficiency is under genetic control and is affected by host and fungal species. Thus, results of the present study clearly indicate that *G. bagyaraji, S. dussii,* and *G. mosseae* did not assist mango in germination. Variation in the nature of mycorrhizal effects on the hosts has been noted by Janos (1980) and Johnson, Graham, and Smith (1997), a concept suggested by Lohman (1927) long ago. Thus, there are notable cases of growth depression apparently caused by the AM fungi in non-host species (Francis and Read 1984) or in host species when phosphate availability is high (Mosse 1973 and Peng, Eissenstat, Graham, *et al*. 1993) or in other cases (Modjo and Hendrix 1986).

Thus, when we consider the inoculation of seed or plants, selection of the right fungal partner is essential to successful and efficient symbiosis. Mango stone germination can be increased by 10% to 15% by inoculation with *Gi. margarita, A. laevis, G. monosporum*, and *G. fasciculatum.*

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New approaches

A new approach for characterization of arbuscular mycorrhiza fungi

Identification of the AMF (arbuscular mycorrhizal fungi) on roots is almost impossible with morphological methods. Due to the presence of contaminating fungi, it is also difficult with molecular biological techniques. To allow broad investigation of the population structure of the AMF in the field, Renker C, Heinrichs J, Kalderf M, and Buscot F (2003) have established a new method to selectively amplify the ITS (internal transcribed spacer) region of most AMF with a unique primer set (*Mycorrhiza* **13**(4): 191–198). Based on available sequences of the rDNA, a primer pair specific for the AMF and a few other fungal groups were designed and combined in a nested PCR (polymerase chain reaction) with the already established primar pair ITS5/ITS4. Amplification from contaminating organism was reduced by an Alu I restriction after the first reaction of the nested PCR. The method was assessed at five different field sites representing different types of habitats. Members of all major groups within the Glomeromycota (except Archaeosporaceae) were detected at different sites. Gigasporaceae also proved detectable with the method, based on cultivated strains.

Centre for Mycorrhizal Culture Collection

User-friendly digital herbaria of arbuscular mycorrhizal fungi for facilitating identification

Reena Singh and *Alok Adholeya*
Centre for Mycorrhizal Research, TERI, Darbari Seth Block, IHC Complex, Lodhi Road,
New Delhi – 110 003, India

Species of the AM (arbuscular mycorrhizal) fungi are currently placed in Zygomycetes in the order Glomales. In the past two decades, there has been an increase in the descriptions of species in the Glomales and in the enormous amount of research on the effects some of these fungi have on the plant growth. The proliferation of species and variation in the standards of descriptions inevitably cause much uncertainty in the minds of the researchers who want to identify their experimental organisms. Unfortunately, the identification of this group of fungi is difficult. The AM fungi are obligate

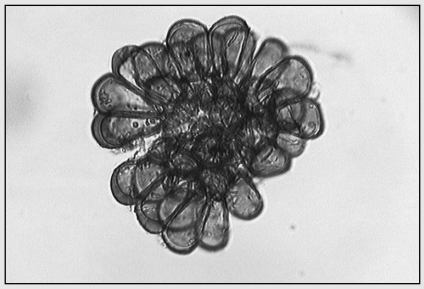
symbionts and cannot yet be cultured on artificial media except for a few species. Their classification is based on the morphology of their resting spores. This implies that for identification purposes, the fungi must have sporulated and that spores must be separated from the substrate or soil in which they started their reproductive phase on a host plant. There is an urgent need for a system to identify or at least, help identify them. Various attempts have been made to devise dichotomous or synoptic keys. The limited use and success of such keys is the result of our limited knowledge of taxonomic character and materials used for description. Thus, any effort to improve the knowledge of taxonomy of these fungi would be an important step. As this field of science is fast losing importance, it becomes the necessity of the taxonomists to exchange and disseminate information about it.

The information can be imparted by either giving formal training to the researchers or by disseminating the collated information on biodiversity. The demand for readily accessible biological expertise is growing. In the scientific community and in society, there is an increasing need for up-to-date information. In the past, scientific data were mainly for scientists. Now the information is needed by a far larger group of users for various purposes, such as pure or applied research, industrial processes, nature conservation, biodiversity inventories, policy-making, etc. In all these cases, it may be vital that the information is up to date and valid. At the same time, the size of the required information sets is growing. We need to know more, update more, and check our data against a bigger variety of the existing information. Last but not least, we are now more in a hurry than ever before. There is a need to access the new data fast as we have to make quick decisions dictated by deadlines of the fast-speeding wheels of time. The ever-growing hunger for information among an increasing number of users all over the globe makes it clear that traditional ways of dealing with information dissemination no longer suffice. The need for publishing scientists' results becomes too slow to meet with our demands; the sheer size of the information is too large to be dealt with on paper, and the information itself (texts, pictures) is too complicated to be dealt with by a printing press.

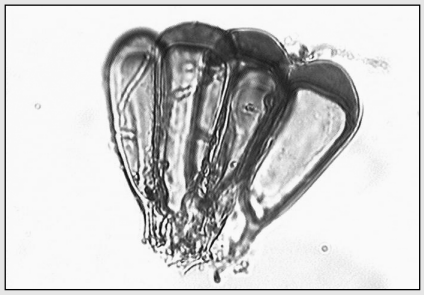
Digital media and electronic data dissemination are fast becoming new instruments for economic and efficient dissemination of our knowledge. The new tools offer exciting possibilities not only for distributing what we know to eager users at large, but also to the science itself and the way we work. Through the media we are able to store and share much more of what we see and experience. Compact discs have become a worldwide-accepted data carrier for digital information.

At the Centre for Mycorrhizal Research, digital herbaria of 34 AM fungal species were prepared. In these herbaria, fact sheets of each species were prepared in which the diagnostic features are listed along with the relevant photographs (see Figure 1 as an example).

Figure 1 Fact sheet of *Sclerocystis clavispora* Trappe



Sporocarps 460–750 mm broad, globose to subglobose, minutely verrucose from exposed tips of spores formed radially in a singly, tightly packed layer around a central plexus of hyphae; base indented



Chlamydopores 140–185 mm, clavate to subcylindric, tapering to a hyphal attachment 70–10 mm diameter. Spore walls 1.5–5 mm thick on the sides, at the spore apex thickened to 17–25 mm, at the base thickened to 5–8 mm and occluding the attachment at maturity.

Improved variety rapeseed mustard: TERI cultivation Uttam–Jawahar

In view of the WTO (World Trade Organization) obligations, the Indian agriculture needs to brace itself to become competitive in the global market through the development of value-added agricultural products. Among the major rapeseed-mustard-growing Asian countries, China is on the way to a change over from high erucic, high glucosinolate-containing traditional varieties to the internationally accepted canola quality cultivars, but such a change over is yet to begin in India.

TERI has taken an initiative in this direction: a broad-based awareness workshop on *Public–Private Partnership: A changeover to value added oilseed in India* was jointly organized by TERI (The Energy and Resources Institute), New Delhi, and JNKVV (Jawaharlal Nehru Krishi Vishwa Vidyalaya) on 26 February 2005. The workshop, co-sponsored by the CSIR/TMOP&M (Council of Scientific and Industrial Research / Technology Mission on Oil, Seeds, Pulses and Maize), Ministry of Agriculture, and Syngenta India Ltd, was well-attended through participation from all stakeholders, comprising scientists, agriculture experts, nutritionists, processing and post-harvest engineers, industrialists, food technologists, farmers, and field extension officers of the Department of Agriculture, who shared their views on various issues pertaining to a change over to value-added oilseeds in view of the post-WTO (World Trade Organization) scenario emerging in India.

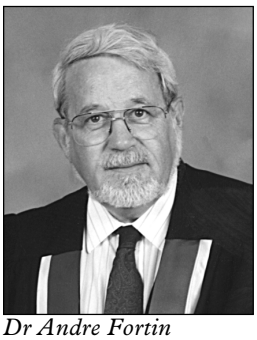
A broad-based awareness workshop on a changeover to value-added oilseeds in India (jointly organized by TERI and JNKVV)

Dr V S Tomar, Director-Research Services, JNKVV, Jabalpur, assisted as Chairman and Dr C B Singh, Dean, College of Agriculture, Jabalpur, acted as co-chairman for all sessions. Dr D P Singh, Vice Chancellor, JNKVV, presided over the plenary session. He appreciated the efforts undertaken for development of nutritionally improved quality variety of rapeseed-mustard, which is one of the few exceptions where varietals improvement has been undertaken for the purpose of domestic consumption as well as making an export-oriented product; this concept should be extended to other crops too. This was further substantiated by Prof. S K Rao, Head, Department of Plant Breeding and Genetics; and Director, Farms, JNKVV, and his fellow scientists.

Dr Abha Agnihotri, Fellow, Plant Biotechnology and Group Leader, Quality Improvement, TERI, New Delhi, presented the success story: *Development of nutritionally improved quality rapeseed-mustard variety TERI Uttam–Jawahar*. Intelligent selection of plants having desired quality parameters as well as good yielding attributes play the most critical part in development of rapeseed-mustard of canola-quality variety. Thus, TERI scientists employed biotechnological approaches to supplement the conventional methods of plant breeding to develop new strains of improved quality, seven of which are registered at the ICAR (Indian Council of Agricultural Research). One of them, (TERI[OO]R9903; INGR No.04077), formed the first double-low variety of *Brassica napus* and is released for cultivation in Madhya Pradesh. Attributes of this high-value crop, based on multi-location testing under the AICRP R&M (All India Coordinated Research Project on Rapeseed-Mustard) of the ICAR, and the data procured at the JNKVV centres and on farm trials in MP (Madhya Pradesh), were enumerated. Dr Agnihotri emphasized that the main focus is not only on developing nutritionally superior quality varieties but also on developing systems to involve farmers, extractors, and promoters/exporters for ultimate benefits to all. Processing engineers were of the view that the existing models of small oil expeller at the village level may be adapted for efficient extraction of oil. Mr Goutia, the Padma Bhushan recipient and farmers' representative was of the view that the workshop has been extremely beneficial to promote the linkage between farmers and scientists. It is therefore very valuable for future betterment of agriculture.

The release of a new variety, *TERI Uttam–Jawahar* having internationally acclaimed canola quality for cultivation across MP is a landmark achievement for the agriculture sector in general, and oil seeds sector in particular, as this variety is rich in oil (approximately 43%) and has high levels of oleic acid (over 60%), which prevents it from getting rancid soon. Moreover, it tolerates pod shattering and white rust, and has shorter crop cycle for the benefit of the farming community. It has proven its suitability to grow in the mustard-growing belts, moreover it has been found to be economical as compared to other crops grown under limited irrigations, and can be an alternative crop in soybean-based cropping systems. *Gobhi Sarson*, which has earlier been restricted to only a few northern states, has now been developed as a versatile crop for the mustard-growing belts of MP. Even with a conservative estimate, the oil and meal together will fetch almost double the price per hectare, and it has good potential in the domestic as well as global markets as a fodder-cum-oilseed-cum oil meal crop for added benefits. The release of superior quality mustard variety was welcomed by all farmers who showed an interest to grow the high-quality oilseed crop from the next season. Establishing linkages for contract farming for an assured marketing system and premium to farmers is being explored for benefits to all stakeholders.

Biography of Dr Andre Fortin


Dr Andre Fortin

Dr Andre Fortin is the world's leading mycorrhizologist actively associated with mycorrhiza research for over the past forty years. Born in 1937 in Quebec, Canada, Dr Fortin did his MSc from the University of Wisconsin in 1964 and PhD from Laval University (Quebec) in 1966. Though now retired, Dr Fortin is presently quite active in the R&D on mycorrhizae. He was scientific chairman for fourth *International Conference on Mycorrhizae* held in 2004. He is presently busy in finalizing the foundation of the International Mycorrhiza Society. Dr Fortin was one of the key scientists who planned and helped in the establishment of Mycorrhiza Network-Asia at TERI in 1988. Out of his varied types of research activities, his five most significant contributions to research on mycorrhizae are highlighted below.

Aseptic cultivation of mycorrhizal fungi on root organ cultures

Dr Fortin (1966) developed an aseptic technique for formation of ectomycorrhiza (ECM) on root-organ culture (PhD thesis). This work became the forerunner in synthesis of the AM (arbuscular mycorrhiza) on genetically transformed carrot roots. Development of this cultivation technique for the AM fungi allowed investigations into many fundamental aspects of the complex biology of this symbiosis, which, according to Dr Fortin, are highlighted in a book by Declerck Strullu and Fortin (in press). These contributions allowed the development of a unique group of industrial products by Premier Tech in Canada and TERI in India, thus making large-scale use of the AM inocula an accessible technology for sustainable agriculture.

The eco-physiological relationship between ectomycorrhizal fungi and conifer trees of boreal forests

Dr Fortin and his team of workers have shown that seasonality of carbon allocation rather than low temperature and high humidity was the primary factor explaining why basidiomes of the ECM fungi are produced in late summer and early fall in boreal forest. They showed that in these regions, the maximum physiological activity (32 p absorption) of mycorrhizal roots was reached by mid-August and that this peak of activity was concomitant with a massive flow of sugars to the roots. This shift in the pattern of carbon allocation from the aerial to the underground parts of the tree followed the establishment of dormancy. Dr Fortin and his team have tested this hypothesis under controlled conditions wherein they were able to demonstrate that it was possible to trigger fruit body development by reducing day length momentarily to induce bud dormancy. When young white pines thus pretreated, were exposed to full sunlight under a long photoperiod and at normal temperatures (that is, without a cold treatment or an excess of nitrogen), they kept producing basidiomata for up to 15 months. The cumulative weight of these fruit bodies was 3.5 times greater than the weight of the host trees. They are currently pursuing R&D on wild edible ECM mushrooms as well as their production under controlled conditions.

Significance of arbuscular mycorrhiza in agriculture

Dr Fortin has conducted research on physiology of the AM and his research group developed the first soil-free AMF inoculum. This permitted pioneering work under field conditions without soil sterilization. The results obtained were largely responsible for recent advances in the R&D made by Premier Tech in the development of inocula for use in horticultural and agricultural production. Dr Fortin is the scientific advisor for this company. In collaboration with C. Planchette, Dr Fortin contributed to a comprehensive paper on the management of AMF in sustainable agriculture.

Role of mycorrhizal fungi in biocontrol of soil-borne plant root diseases

One of the first studies of Dr Fortin and his colleagues on mycorrhizal fungi as biocontrol agents was on the relationship between ectomycorrhizal fungi and the nematode, *Aphelenchus avenae*. The studies revealed that several of the ECM fungi allowed the growth and reproduction of the nematode. *Rhizopogon roseolus*, however, proved to be highly toxic, killing the nematode within minutes. These investigations were followed by the demonstration of antibiotic production that inhibited growth of several pathogenic fungi leading to the identification of two active substances. Investigations on the AMF demonstrated that these fungi protect plant roots against disease. The mechanism is not only due to the triggering of plant defence mechanism but also due to the reduction in the number of propagules of pathogenic fungi in the rhizosphere even before they contact the roots.

Synergism between AM fungi and PSB (phosphate solubilizing bacteria)

Dr Fortin and his colleagues demonstrated under aseptic conditions using the split-plate method that neither the AM fungus, *Glomus intraradices,* nor the bacteria, *Pseudomonas aeruginosa,* were able to individually solubilize tricalcic phosphorus. They were, however, able to do so in concert. It was also shown that either ammonium on nitrate could be used for this activity. Also, pH changes could not explain the results. Dr Fortin observed for the first time, the formation of biofilm on extramatrical AMF hyphae. Currently, R&D is being pursued on the presence, species composition, and functions of bacterial biofilms on the surface of the AM fungal mycelium under controlled laboratory conditions.

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.

- *Applied Soil Ecology*
- *Canadian Journal of Botany-Revue Canadienne De Botanique*
- *Canadian Journal of Plant Science*
- *Ecological Applications*
- *Ecology Letters*
- *Environmental Studies*
- *Fems Microbiology Letters*
- *Folia Microbiologica*
- *Forest Pathology*
- *Functional Ecology*
- *Geoderma*
- *Journal of Applied Botany and Food Quality-Angewandte Botanik*
- *Journal of Ecology*
- *Journal of Plant Physiology*
- *Microbial Ecology*
- *Mycotaxon*
- *Nature*
- *New Phytologist*
- *Phytochemistry*
- *Plant and Soil*
- *Plant Journal*
- *Plant Soil and Environment*
- *Soil Science and Plant Nutrition*

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 journal, volume no., issue no., page nos [corresponding address given for author whose name is highlighted in asterisk]
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FORM IV
(as per Rule 8)

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Signature of Publisher

Date: 1 April 2005

Dr R K Pachauri

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3 May 2005

56th International Symposium on Crop Protection
K De Jonghe, Department of Crop Protection, University of Gent, Coupure Links 653, B–9000 Gent, Belgium

Fax +32 9 264 6 238 • *E-mail* Kris.DeJonghe@rug.ac.be • *Tel.* +32 9 264 6 022

Gatersleben, **Germany**
3–6 June 2005

Eighth Gatersleben Research Conference on *Genetic Diversity and Genome Dynamics in Plants*
Ms Waltraud Mühlenberg, IPK (Institut für Pflanzengenetik und Kulturpflanzenforschung), Correnssstraße 3, 06 466 Gatersleben, Germany

Fax +49 394825500 • *E-mail* grc2005@ipk-gatersleben.de
Website http://www.ipk-gatersleben.de

Rochester, Minnesota, **United States**
12–15 June 2005

Ninth North American Agroforestry Conference
Organized by Association for Temperate Agroforestry

E-mail <curre002_@_umn.edu>, <keef0039@umn.edu>
Website http://cinram.umn.edu/afta2005/

Philadelphia, **USA**
19–22 June 2005

BIO 2005 Annual International Convention
BIO Conventions & Conferences, 1225 Eye Street, NW Suite 400, Washington, DC 20005

Fax 202 589 2545 • *E-mail* bio2005@bio.org • *Tel.* 202 962 6655

Saltillo Coahuila, **Mexico**
26–30 June 2005

Tenth International Symposium on Plant Bioregulators in Fruit Production
Dr Homero Ramirez, Salazar 1081, Zona Centro, Saltillo Coahuila 25000, Mexico

E-mail homeror@terra.com.mx • *Website* www.saltillo2005.org • *Tel.* + 52 8417 4167

Aberystwyth, **UK**
3–7 July 2005

The fourth International Symposium on the Molecular Breeding of Forage and Turf
IGER, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK

Fax +44 0 1970 82 3241 • *E-mail* mervyn.humphreys@bbsrc.ac.uk
Tel. +44 0 1970 82 3170 • *Website* www.iger.bbsrc.ac.uk/igc/welcome.htm

Delft, **The Netherlands**
3–8 July 2005

Biotrans 2005: Seventh International Symposium on Biocatalysis and Biotransformations
Biotrans 2005 Secretariat, Department of Biotechnology, Julianalaan 67, 2628 BC Delft, The Netherlands

Fax +31 15 278 2355 • *E-mail* biotrans2005@tnw.tudelft.nl
Tel. +31 15 278 2342 • *Website* http://www.biotrans2005.bt.tudelft.nl/

Seattle, Washington, **USA**
16 July 2005

Plant Biology 2005
American Society of Plant Biologists, 15501 Monona Drive, Rockville, MD 20 855–2768, USA

Fax +1 301 279 2996 • *E-mail* info@aspb.org
Tel. +1 301 251 0560 • *Website* http://www.aspb.org/meetings/pb-2005/index.cfm

SECC, Glasgow, **UK**
17–21 July 2005

BioScience2005: From genes to systems

Fax +44 0 20 7637 7626 • *E-mail* meetings@BioScience2005.org
Tel. +44 0 20 7580 3481 • *Website* www.bioscience2005.org

Cancún, **México**
17–22 July 2005

International Congress on Molecular Plant–Microbe Interactions
Amy Hope, IS-MPMI 3340, Pilot Knob Road, St. Paul, MN 55 121-2097, USA

Fax +1 651 454 0766 • *E-mail* ahope@scisoc.org
Tel. +1 651 454 7250 • *Website* www.ismpminet.org/

San Francisco, California, **USA**
23–28 July 2005

XI International Congress of Bacteriology & Applied Microbiology (IUMS 2005)

E-mail IUMS@asmusa.org

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