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## About TERI

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

## The Bioresources and Biotechnology Division

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

## The Mycorrhiza Network and the Centre for Mycorrhizal Culture Collection

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

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

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



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## Role of mycorrhiza in disturbed lands. Part II. Soil compaction, soil erosion, soil aggregation, and volcanic eruptions

*Sujan Singh*\*

TERI, Darbari Seth Block, India Habitat Centre, Lodhi Road, New Delhi – 110 003, India

### Effect of soil compaction on mycorrhiza

Soil compaction results usually from treading by cattle, sheep, goats, etc. during grazing, absence of soil working for prolonged periods, movement of vehicles and heavy machinery, use of land as playgrounds, etc. Soil compaction impedes air circulation and water percolation into the subsoil surfaces, affecting the activity of mycorrhizal fungi.

### Effect of soil compaction on VAM

In studies conducted at the Department of Forestry, Purdue University, West Lafayette, Indiana, USA, seedlings of sweet gum (*Liquidamber styraciflua*) and yellow poplar (*Liriodendron tulipifera*) were transplanted into pots that contained silt-loam soil compacted to bulk densities of 1.25, 1.40 or 1.55 Mg m<sup>-3</sup>. Chlamydo spores of *Glomus macrocarpum* or *Glomus fasciculatum* were used to inoculate seedlings. In control plants filtrates were used to inoculate the seedlings. The weight and length of yellow poplar roots were significantly greater at lower bulk densities but the fibrosity of the root system was unaffected by increasing bulk density. Weight, length, and fibrosity of the sweet gum root system decreased significantly with each increase in the bulk density. Inoculated yellow poplar seedlings had greater root weights at each bulk density than noninoculated seedlings, but root length was not influenced by mycorrhizal treatment at high bulk densities. Fibrosity of yellow poplar roots varied with mycorrhizal treatment at each bulk density. The results thus indicate that for yellow poplar, compaction effects may not outweigh mycorrhizal benefits at higher bulk densities. At each bulk den-

sity, sweet gum seedlings inoculated with *Glomus fasciculatum* showed the greatest root growth, suggesting that the effects of compaction could be alleviated in sweet gum by inoculation with this mycorrhizal fungus (Simmons and Pope 1987).

Studies conducted at the Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma, USA on responses of *Schizachyrium scoparium* and its mycorrhizal symbionts to clipping and compaction showed that all treatment combinations significantly reduced the growth and biomass of plants relative to controls. Compaction significantly reduced tillering and crown expansion while clipping increased tillering early in the growing season and reduced it later. Mycorrhizal colonization of roots was highest in the clipped plots and lowest in the compacted plots while spore number was highest in the compacted plots and lowest in the clipped plots. Spore number may thus be negatively correlated with root growth since any treatment that reduced plant growth yielded higher spore numbers (Wallace 1987).

In studies conducted at the Central Institute of Medicinal and Aromatic Plants, India, plants of *Citronella java* (*Cymbopogon winterianus*) in a pasteurized sandy loam soil were inoculated either with rhizosphere microorganisms excluding VAM fungi (non-mycorrhizal) or with VAM fungus, *Glomus intraradices* (mycorrhizal) and supplied with 0, 50, and 100 mg P per kg soil. The soil was compacted to bulk densities of 1.2 and 1.4 Mg per cubic metre (dry soil basis). *G. intraradices* substantially increased root and shoot biomass, root length, nutrient (P, Zn, and Cu) uptake per unit root length, and nutrient concentration in the

\* Compiled from TERI database – RIZA

plants as compared to inoculation with rhizosphere microorganisms when the soil was at low bulk density and not amended with P. Little or no response to the VAM fungus was observed when the soil was supplied with 50 or 100 mg P per kg soil and/or compacted to the highest bulk density. At higher soil compaction densities and P supply, VAM inoculation significantly reduced root length. Non-mycorrhizal plants with higher soil compaction produced relatively thinner roots and had higher concentrations and uptake of P, Zn, and Cu than at lower soil compaction, particularly under conditions of P deficiency. The quality of *C. java* oil measured in terms of citronellol and d-citronellol concentration did not vary appreciably with soil treatment (Kothari and Singh 1996).

In studies conducted at the Department of Plant Nutrition, China Agricultural University, Beijing, China, the influence of *Glomus mosseae* on the effects of soil compaction on growth and phosphorus uptake of red clover (*Trifolium pratense*) was studied in three-compartment pots; the central one with a soil bulk density of 1.3 g per cubic centimetre and the other compartments with soil densities of 1.3, 1.6, and 1.8 g per cubic centimetre. The soil in the outer compartments was fertilized with phosphorus and was either freely accessible to both, roots and fungal hyphae, or separated by nylon mesh and accessible to the hyphae only. At a soil bulk density of 1.3 g per cubic centimetre, mycorrhizal plants did not absorb more phosphorus than non-mycorrhizal plants except when nets restricted the access of roots to the outer compartments. At high soil bulk density, root growth drastically decreased. Hyphae of *Glomus mosseae*, however, absorbed phosphorus even from highly compacted soil, and induced a phosphorus depletion zone about 30 mm from the root surface. Consequently, at the higher soil bulk density, shoot phosphorus concentration and the total amount of phosphorus in the shoot were higher in mycorrhizal than in the non-mycorrhizal plants. This shows that the hyphae of *G. mosseae* are more efficient in obtaining phosphorus from compacted soil than mycorrhizal or non-mycorrhizal roots of red clover (Li, George, Marschner *et al.* 1997).

### *Effect of soil compaction on ectomycorrhiza*

In studies conducted at the Rocky Mountain Research Station, Moscow, Idaho, USA, two levels of soil compaction (none and severe) and a stump extraction on an ash cap soil were done and the treated soils were planted with Douglas fir (*Pseudotsuga menziesii* var. *glauca*) and western white pine (*Pinus monticola*) seedlings. Soil compaction increased post harvest bulk density by 15–20 per cent to a depth of 30 cm. Stump removal decreased surface soil bulk density but it increased bulk density at 30–45 cm depth to levels

equal to soil compaction treatment. One year after treatment, seedling top weights were similar among treatments but root volume was significantly reduced in the soil compaction treatment. Soil compaction and stump removal treatments also reduced the numbers and morphological types of ectomycorrhizae in Douglas fir and western white pine (Page-Dumroese, Harvey, Jurgensen *et al.* 1998).

In similar studies conducted at the United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, Grants Pass, Oregon, USA, Douglas fir and western white pine were subjected to treatments including organic matter removal, mechanical soil compaction (no compaction, moderate compaction, and severe compaction) following outplanting. Moderate and severe soil compaction significantly reduced non-mycorrhizal root tip abundance in both species. Ectomycorrhizal root tip abundance and ectomycorrhizal diversity were significantly reduced in Douglas fir seedlings in severely compacted areas (*Amaranthus*, Page-Dumroese, Harvey *et al.* 1996).

### *Effect of erosion on mycorrhiza*

Erosion of surface soil washes away that part of soil which has the most intense biotic activity. Surface soil is rich in the inocula of mycorrhizal fungi and its removal affects the inoculum potential of the mycorrhizal fungi.

### *Effect of surface soil removal on mycorrhizal efficiency*

Studies conducted at the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii, USA, on an Oxisol showed that the density of VAM infective propagules diminished as the level of simulated erosion (removal of surface soil) was increased from 0–50 cm. Surface soil removal not exceeding 7.5 cm was associated with decreased propagule abundance without adverse effect on VAM colonization of roots and the symbiotic effectiveness of the VAM fungi. The extent of VAM colonization of the roots and the degree of symbiotic effectiveness observed at this level of simulated erosion were significantly higher than those observed in the soil not subjected to simulated erosion. This stimulation is attributed to the removal of antagonistic biotic factors from the top 7.5 cm of soil removed, which is rich in such organisms. Simulated erosion in excess of 7.5 cm of surface soil removal was generally associated with a significant decrease in the number of total and active VAM propagules. It also significantly delayed the development of VAM effectiveness monitored in terms of the P status of *Leucaena leucocephala* subleaflets, and curtailed the level of maximum observed effectiveness. Decreases in VAM effective-

ness were significantly correlated with decreases in the soil chemical constituents. However, the level of infection on *L. leucocephala* roots observed at harvest was not significantly influenced by simulated erosion unless the removal of surface soil exceeded 25 cm (Habte, Fox, and El-Swaify 1988; Habte 1989).

### *Effect of VAM inoculation on eroded soil*

Studies conducted at the University of Hawaii, Honolulu, Hawaii, USA, showed that the extent of colonization of *Leucaena leucocephala* roots increased significantly due to VAM inoculation of Oxisol subjected or not, to simulated erosion. The highest level of colonization of *L. leucocephala* occurred with inoculation of *Glomus aggregatum*, followed by *G. mosseae* and *G. etunicatum*. Increased infection associated with inoculation of eroded soil did not result in enhanced mycorrhizal effectiveness. In similar studies with *Vigna unguiculata* on an oxisol subjected to simulated erosion, inoculation of eroded soil with the above three fungi resulted in increased VA-mycorrhizal colonization of roots without enhancing root P concentration and dry matter yields. Inoculation of uneroded soil, however, led to a significant improvement in root colonization and symbiotic effectiveness in both *L. leucocephala* and *V. unguiculata*. The lack of expression of mycorrhizal effectiveness in eroded soil appeared to be a result of nutrient deficiency (Aziz and Habte; 1989b, 1989c).

### *Effect of ectomycorrhizal inoculation on eroded soil*

In studies conducted at the Department des Science Forestieres, Faculte de Forestierie, University of Laval, Saint-Foy, Quebec, Canada, one month old jack pine seedlings produced in growth pouches and inoculated or not with ectomycorrhizal fungi, were outplanted in diverse stations and later excavated for assessing mycorrhizal colonization. Non-mycorrhizal control seedlings showed 0, 20, 20, and 76 percent mycorrhizal development in sterilized denuded, unsterilized denuded, burnt, and undisturbed jack pine stands, respectively. Based on indices of colonization and competition, *Laccaria bicolor* was the best colonizer at all the stations except at the undisturbed jack pine stands, where *Rhizopogon rubescens* was the best colonizer and also the most competitive. *Pisolithus tinctorius* was not competitive with the indigenous mycota at the burned or the undisturbed jack pine stand stations (McAfee and Fortin 1986).

### *Effect of phosphorus amendment on mycorrhiza in eroded soil*

Studies were conducted at the Department of Agronomy and Soil Science, University of Hawaii, Honolulu, Hawaii, USA, to monitor the VAM de-

velopment in terms of phosphorus status of leaf discs in *Vigna unguiculata* grown in a typical Oxisol which was subjected to simulated erosion. VAM activity was not detected in the eroded soil unless the soil was amended with phosphorus. When phosphorus was not limiting, VAM activity (effectiveness) was detected as early as 17 days from planting, the activity peaking 5–10 days thereafter. Peak VAM activity was observed in a soil solution phosphorus level of 0.026 mg per ml and the peak values were similar in eroded and uneroded soil samples. The maximum mycorrhizal inoculation effect was also observed at this level of soil solution phosphorus. In studies with *Leucaena leucocephala* also, it was observed that VAM effectiveness in a soil subjected to 30 cm of surface soil removal was not restored to a significant extent unless the soil was amended with P, even though the other nutrients were restored to sufficiency level (Aziz, Habte 1987; Habte, Fox and El-Swaify 1988).

### *Effect of nitrogen amendment on mycorrhiza in eroded soil*

Studies were conducted at the University of Hawaii, Honolulu, Hawaii, USA, on *Leucaena leucocephala* grown in pots in an Oxisol subjected to simulated erosion, inoculated with *Glomus aggregatum* and amended with 0, 25, 50, 50, and 100 ppm N. The extent of VAM colonization of roots increased with increasing levels of N in both the eroded and uneroded soils. However the level of infection was significantly higher in the eroded soil than in the uneroded soil. Mycorrhizal activity monitored in terms of P content of *Leucaena leucocephala* sub leaflets increased significantly in the eroded soil with 25 ppm N and became similar to that observed in uneroded soil. Nodule dry matter and shoot N concentration increased significantly with N application up to 50 ppm. Above this level of N, nodule dry weight declined while N concentration did not change. Application of 25 ppm N to the eroded soil also significantly increased shoot and root dry weights while no change was observed in uneroded soil. Further increase in N level did not improve the yield (Aziz and Habte 1989a).

In similar studies conducted at the above University on *Vigna unguiculata* on an Oxisol, subjected or not to simulated erosion, plants were grown in pots containing soil inoculated with *Glomus aggregatum* and amended with 0–100 mg N per kg soil. VAM colonization of roots, shoot P and N status and shoot, root and nodule dry weights were significantly stimulated by N amendment of eroded soil but the response in the uneroded soil of these parameters was not significant. The relationship of N with root colonization and shoot P status was described by a linear model while the relationship between N and the other variables measured was described by quadratic equations (Aziz and Habte 1990a).

### *Effect of basal nutrients, P and N amendments on mycorrhiza in eroded soil*

In studies conducted at the University of Hawaii, Honolulu, Hawaii, USA, cow pea (*Vigna unguiculata*) and *Leucaena leucocephala* in two separate experiments were grown in an Oxisol, subjected or not to imposed erosion, with or without *Glomus aggregatum*, with or without a basal nutrient consisting of K, Mg, S, Zn, Cu, and B and with basal nutrients plus lime, N and P. The extent of mycorrhizal colonization and effectiveness, measured in terms of leaf/sub leaflet discs P content, increased significantly when the eroded soil was amended with a combination of all nutrients and inoculated with *G. aggregatum*. VAM inoculation and nutrient amendment were also accompanied by significant increases in shoot P, Cu, Zn, and N (in cow pea only) and nodule, shoot and root (in cow pea only) dry matter yields. In *L. leucocephala*, it was found that P was the most important nutrient, limiting mycorrhizal effectiveness in the eroded soil and this was followed by N and lime (Aziz and Habte 1990b; Habte and Aziz 1991).

### *Effect of manure or organic amendments on mycorrhiza in eroded soil*

In studies conducted at the Brigham Young University, Department of Agronomy and Horticulture, Provo, Utah, USA, eroded or levelled silt loam soils had been restored to top soil productivity levels by manure application but not by other organic sources such as cheese whey. In a greenhouse experiment on dry bean (*Phaseolus vulgaris*), roots grown on subsoil treated with manure or composted manure showed greater mycorrhizal colonization than roots on untreated subsoil but roots in topsoil had the highest colonization. Topsoil promoted the greatest percent colonization in early bean growth and this was reflected in greater Zn uptake during the early stages of growth. By day 56, plants grown in manured subsoil absorbed Zn equal to the topsoil and at higher levels than the subsoil control. However, this increase in Zn uptake was not seen in plants grown in composted manured subsoil. A decrease in root and shoot weight was observed in composted manured subsoil. Overall mycorrhizal colonization was less than 5%, 21 days after planting and it increased to 58% by 56 days (Tarkalson, Jolly, Robbins *et al.* 1998a).

In a further field study at the above University on wheat (*Triticum aestivum*) and sweet corn (*Zea mays*), mycorrhizal root colonization was higher with untreated (treated with conventional fertilizers) than with dairy manure treated wheat and sweet corn. Also, root colonization was higher in subsoil than in topsoil for wheat but there was no difference between soils for sweet corn. Yield of wheat was highest for manure treated sub soil and topsoil compared to untreated soil (Tarkalson, Jolly, Robbins *et al.* 1998b).

### **Effect of grazing on mycorrhiza**

Studies conducted at the United States Department of Agriculture, Agriculture Research Service, Forage and Livestock Research Laboratory, Oklahoma, USA, on winter wheat subjected or not to autumn grazing, and examined during the following spring showed that root colonization with VAM occurred in both grazed and ungrazed winter wheat during reproductive growth (late spring). Prior to May, low soil temperature apparently inhibited VAM colonization. Percent VAM colonization did not differ between treatments during reproductive growth but root length per unit of soil volume was reduced by grazing resulting in a greater colonized root length for ungrazed plants. Grazing reduced the total carbohydrate pool. Although grazing and VAM colonization were separated in time, a grazing effect on colonization was evident. Colonization may be governed by the ability of wheat plants to recover from grazing during the period prior to VAM spore germination. Although grazed plants gained more carbon during reproductive growth, the gain was not sufficient to overcome the grazing induced carbon limitation as grain yields and VAM root length density were reduced by 16% and 38%, respectively (Trent, Wallace, Svejcar *et al.* 1988).

### **Effect of mycorrhiza on soil aggregation**

Physical entanglement of plant roots and hyphae of mycorrhizal fungi are known to be involved in the binding of micro aggregates with macro aggregates. The process of this soil aggregation may be enhanced by the secretion of chemicals by hyphae of mycorrhizal fungi.

### *Soil aggregation by mycorrhiza*

Studies conducted at the Department of Agriculture, Agriculture Research Service, Horticultural Crops Research Laboratory, Corvallis, Oregon, USA, showed that in pot experiments with peas, an isolate of *Glomus mosseae* did not significantly affect seed yield (8%) but improved soil aggregation by 400% in a gray silt loam soil, high in organic matter (OM) and phosphorus. In another soil, a yellow clay loam, low in OM and phosphorus, seed yield was significantly enhanced (57%) but there was only a small change (50%) in aggregation. It is thus concluded that this fungus influenced the carbon allocation between the plant (measured as seed yield) and the soil (measured as formation of water stable aggregates). The soil appeared to gain carbon at the expense of carbon lost by the plant. Mycorrhizal fungi thus seem to affect the biologically controlled aspects of sustainable agriculture i.e., plant production and soil quality (Bethlenfalvay and Barea 1994).

Studies conducted at the McGill University, Department of Natural Resource Science, Quebec, Canada, on leek (*Allium porrum*) showed that the

abundance of soil aggregates in the 0.5–2.0 mm diameter range was positively related to response to inoculation with *Glomus intraradices* and *Glomus versiforme* at low phosphorus levels but negatively related to high phosphorus levels (Hamel, Dalpe, Furlan *et al.* 1997).

In studies conducted at the University of Western Australia, Faculty of Natural and Agricultural Science, Nedlands, Western Australia, increased length of mycorrhizal hyphae was encouraged by inoculation of *Lolium rigidum* with *Scutellospora calospora* and the effects of roots on soil aggregation was restricted by containment within 38 µm mesh bags. The hyphae of saprophytic fungi were encouraged by the addition of glucose or straw substrates at 2.3 mg per g. After incubation of the soil for five weeks, changes in 72 mm water stable soil aggregates was assessed in root-free soil before and after dry sieving through an 8 mm mesh to test the resistance of aggregates to soil abrasion. Hyphal lengths and hot water extractable carbohydrate C content was measured in the 72 mm water stable fraction and non-aggregate (<2 mm) fraction of the soils that was not dry sieved. The treatments increased hyphal lengths from 3.2 m per g to 4–6.2 m per g but the hyphal lengths were not different between the treated soils because much of the hyphal growth occurred only in the soil that was aggregated. The length of the hyphae in the 72 mm aggregates from straw and glucose amended soils was 2–5.7 m per g greater than the length in the non-aggregated soil (4–6 m per g). Inoculation of the non-amended soil increased the proportion of mycorrhizal roots by 340% but had little effect on hyphal length. Water stable aggregation increased from 7 to 16 g per kg in the non-amended soils and 84 to 143 g per kg in the straw and glucose amended soil (Degens, Sparling, and Abbott 1996).

Studies conducted at the Estacion Experimental del Zaidin, Consejo, Superior de Investigaciones Cientificas, Granada, Spain, on *Pisum sativum*, grown on a gray silt loam of high phosphorus and a yellow clay loam of low phosphorus content showed that without VAM inoculation, both soils disaggregated. The slaking of water-stable aggregates (more than 0.5 mm) was significantly less severe when rhizobacterium was present. With VAM fungus, soil aggregation increased by upto 27% during the experiment (Andrade, Azcon, and Bethlenfalvai 1995).

In an experiment conducted at Denmark, *Sorghum bicolor* was grown for ten weeks in soil, pasteurized or un-pasteurized, uninoculated or inoculated with AM fungi, to see the effect of AM fungi on the aggregate stability of a semi-arid Indian vertisol. Soil exposed to the growth of roots and hyphae (outside mesh bags used to exclude roots and allow hyphae to enter) showed aggregates with a larger geometric mean diameter (GMD) in inoculated pasteurized soil than in uninoculated

pasteurized soil. There were no significant differences in GMD between inoculated pasteurized soil and un-pasteurized soil. No significant effects of inoculation on plant growth were found in pasteurized soil exposed to hyphal growth only. However, un-pasteurized soil had significantly higher GMD than pasteurized inoculated or uninoculated soil with or without plant roots. Turbidimetric measurement of soil exposed to roots and hyphae showed the highest aggregate stability for the inoculated pasteurized soil (Bearden and Petersen 2000).

### *Soil aggregation with VAM and associated host plants*

Studies conducted at the Department of Soil Science, Waite Agricultural Research Institute, University of Adelaide, Glen, Osmond, South Australia, showed that the root system of rye grass was more efficient than that of white clover in stabilizing aggregates of Lemnos loam soil because rye grass supported a larger population of VAM fungal hyphae in the soil (Tisdall and Oades 1979).

Further studies conducted at the above University showed that the quickest way to stabilize aggregates in pot soil from 0–10 cm layer of red brown earth was to grow rye grass with ample water and to clip the tops at monthly intervals. Clipping appears to stimulate the growth of VAM fungal hyphae. Stressing the plants by allowing them to wilt reduced the stability of the aggregates. The mycorrhizal hyphae persisted for at least several months after the plants had died. Although the hyphae may not have been viable, they continued to bind particles of soil in stable aggregates (Tisdall and Oades 1980a).

Studies were conducted at the Department of Horticulture, Pennsylvania State University, Pennsylvania, USA, to observe the influence of cover crops, winter wheat (*Triticum aestivum*) and a perennial weed, dandelion (*Taraxacum officinale*), on AM inoculum potential, soil aggregation, and maize yield after one season. Winter wheat and dandelion both improved soil aggregation. Mycorrhizal colonization of maize was higher with cover crops when compared with fallow (Kabir and Koide 2000).

Some studies, however, showed that in some forage crops the VAM fungi do not play any role in soil aggregation. Growth room pot experiments were conducted at the Research Station Agriculture, Harrow, Ontario, Canada, to relate differences in the aggregating ability of several grasses and legumes to root development and frequency of VA-mycorrhizal fungi. Forages with most extensive root development within 80 days resulted in the greatest improvements in aggregation. Although the frequency of VA mycorrhiza varied between forages, it was not associated with improvements in aggregation (Stone and Buttery 1989).

### *Effect of VAM on soil aggregation in association with organic matter*

Studies conducted at the Department of Soil Science, Waite Agricultural Research Institute, University of Adelaide, Osmond, South Australia, showed that fifty years of crop rotations had decreased the stability of micro aggregates (250  $\mu\text{m}$  diameter) of Urrbrae fine sandy loam soil and simultaneously decreased the lengths of roots and hyphae of mycorrhizal fungi and also the total organic matter in the soil. Regardless of the rotation, particles 50–250  $\mu\text{m}$  diameter were stable and were stabilized by organic matter (Tisdall and Oades 1980b).

### *Effect of VAM on soil aggregation in shifting sand dunes*

Studies conducted at the Centre for Investigation Biology, Noroeste, Baja California, Mexico, showed that in a desert ecosystem, occurrence of soil mounds under plants owing to soil deposition was related to the nature of plant canopies and to the VAM status of the roots. Mound soils were enmeshed with VAM fungal hyphae, especially in the upper layer (approximately 10 cm). Seedlings of *Pachycereus pringlei* (a cactus), growing in a screen house for six months in soil collected from under *Prosopis articulata* had a biomass ten times greater than plants growing in a bare-area soil. The results showed that the VAM fungi helped to stabilize wind-borne soil that settled under dense canopies, enhanced the establishment of colonizer plants in bare soils of disturbed areas and influenced plant associations through differences in the mycotrophic status of the associates (CarrilloGarcia, de la Luz, Bashan *et al.* 1999).

Field experiments were conducted at the Agricultural University, Norway Department of Biology, Norway, on *Schizachyrium scoparium* (a dominant perennial bunch grass member of *Quercus havardii* (sand shinnery oak), communities of semiarid western Texas) grown mounds of displaced soil produced by rabbit (*Sylvilagus audubone*), off mound soil and artificially created mounds. The results showed that soil mound characteristics increased seedling survival, shoot and root biomass, root lengths, number of tillers, mycorrhizal infection and nutrient uptake more in plants grown on mounds than off mounds. Artificial mounds generated from soils associated with herbaceous communities were more similar to intact rabbit mounds than artificial mounds generated from soil associated with the oaks (Dhillion 1999).

### *Mechanism of soil aggregation by mycorrhiza*

Soil aggregation by VAM fungi may involve physical entanglement of hyphae of VA-mycorrhizal

fungi and the finer roots of the host plants or may be effected by the production of some chemicals by the hyphae of VAM fungi, which bind the soil particles. Studies conducted at the Environmental Research Division, Argonne National Laboratory, Illionis, USA, by using data collected from soils of a chronosequence of tall grass prairie restoration and an adjacent prairie soil cultivated with row crops for at least 100 years showed that root lengths by diameter size classes, the length of roots colonized by mycorrhizal fungi with in each root size class and extraradical hyphal lengths of mycorrhizal fungi were all highly correlated with the geometric mean diameter (GMD) of water-stable soil aggregates. Path Analysis showed that extraradical hyphal length followed by fine root length (0.2–1.0 mm diameter) had the strongest direct effect on GMD. It was expected that a physical entanglement mechanism would involve the very finest roots; however the direct path between very fine root lengths (0.2 mm diameter) and GMD was not significant. Although both root size classes exhibited significant indirect effects on GMD via the relationships between their colonized root lengths and extraradical hyphal lengths, the overall effect of fine root length on GMD was much stronger than the effect of very fine root lengths (Willer and Jastrow 1990).

In further studies conducted at the above laboratory, a conceptual model of the relationships between selected biotic factors and soil aggregation in a chronosequence of restored tall grass prairie was evaluated by using the statistical technique of path analysis. External VA-mycorrhizal hyphae, roots with diameters of 0.2 mm and microbial biomass carbon exerted the strongest direct effects on the percentage of water-stable soil microaggregates, 0.212 mm in diameter. Roots with diameters of between 0.2 and 1.0 mm exerted a very strong indirect effect on aggregation via their contributions to external mycorrhizal hyphae, microbial biomass carbon and hot-water soluble carbon. When microaggregates of different soil classes were evaluated by this technique, the relative importance of external hyphae, roots, microbial biomass carbon, hot water soluble carbon, and soil organic carbon varied. In general, external hyphae and hot water soluble carbon were most important in microaggregates of 2 mm diameter. The roles of microbial biomass carbon, roots (0.2 mm diameter) and soil organic carbon were greatest for smaller microaggregates (Jastrow and Michael 1993).

Studies conducted at the Department of Soil Science, Waite Agricultural Research Institute, University of Adelaide, South Australia, on stabilization of soil aggregates by rye grass and white clover showed that the hyphae of VAM fungi, as seen in electron micrographs, were covered with a layer of amorphous material, probably polysaccharides, to which clay particles appeared firmly attached (Tisdall and Oades 1979).

Studies conducted at the United States Department of Agriculture, Agricultural Research Science, Soil Microbiological Systems Laboratory, Agricultural Research Center, Beltsville, Maryland, USA, on production of extracellular compounds by VAM fungi in soil and their role in soil aggregation indicated that the crude extracts of protein from hyphae and the soil showed the same banding patterns and density of bands on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Enzyme-linked immunosorbent assay (ELISA) values for soils were between 60 and 107 percent of the hyphal ELISA value. Total protein concentration was correlated linearly with organic carbon in soil. The percent dry weight of soil composed of water stable aggregates correlated positively with silt and ELISA values and correlated negatively with sand (Wright and Upadhyaya 1996).

In further studies conducted at the above Center, a comparison was made between concentrations of glomalin, a glycoprotein produced by AM fungi, and soil aggregate stability. The stability was measured on air-dried aggregates re-wetted by capillary action and then subjected to wet sieving for ten minutes. The monoclonal antibody, which is used to discover glomalin in AM fungi, was employed to assess immunoreactive glomalin in soil aggregate surfaces by immunofluorescence and in extracts from aggregates by ELISA. Immunofluorescence was most evident on aggregates with high glomalin concentration though it was observed on at least some surfaces of aggregates for all soils examined. Aggregate stability was linearly correlated ( $P > 0.001$ ) with all measures of glomalin (mg per g of aggregates) in these soils (Wright and Upadhyaya 1998).

Further studies conducted at the above Center on maize showed that during the first three years in transition from plough tillage (PT) to no tillage (NT), there was a high linear correlation ( $r^2 = 0.78$ ,  $n = 32$ ) between glomalin (a glycoprotein produced by AM fungi) concentration in aggregates and aggregate stability. Increases in both aggregate stability and glomalin were measurable from year to year in NT plots but the values for NT was significantly higher than those for PT after 2–3 years ( $P > 0.05$ ). Comparison of NT plots after three years with nearby soil in grass cover indicated that the stability was greater by 20% and glomalin concentrations higher by 45% in the grass covered soil. Comparison of PT and NT (3 years) inter row samples with intra row samples indicated that the plant roots and NT management may have a synergistic effect on aggregate stabilization (Wright, Starr, and Paltineanu 1999).

Studies conducted at the University of Western Australia, Faculty of Agriculture, Nedlands, Australia, on relations between soil aggregates and length of hyphae of mycorrhizal fungi showed that under a scanning electron microscope, sand grains in the aggregates appeared to be linked together

only by hyphae. Hot water extractable carbohydrate C contents of water stable aggregates and non-aggregate soils were not different indicating little involvement of microbial polysaccharides in stabilizing the aggregates. Despite the strong evidence of hyphal involvement in stabilizing aggregates, only the hyphal lengths in the whole non-amended soil and 72 mm aggregate fraction of the soil amended with glucose were significantly correlated with aggregation. The amounts of dry stable aggregates in 8 mm sieved soil were generally increased by 63%–147% in the amended soil compared to non-amended soil, and 27%–33 % of aggregates were water stable indicating that the hyphae also contributed to the stabilization of dry aggregates (Degens, Sparling, and Abbott 1996).

### *Interactions among mycorrhiza, soil aggregates, and rhizobacteria*

In experiments conducted at the USDA-ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon, USA, in four-compartment soil containers, roots of *Sorghum bicolor* plants were split over the entire barrier and the roots on one side were inoculated with a VAM fungus. Two compartments on each side of the solid barrier were separated by a 43  $\mu\text{m}$  screen that permitted the passage of the hyphae but not of the roots. The design produced mycorrhizosphere soils (M) by AM roots or hyphosphere (H) soils by the AM hyphae in the two compartments on one side of the barrier and rhizosphere soil (R) by non-AM roots or root or hyphal free bulk soil (S) in the two compartments on the other side. At harvest (ten weeks), there were significant differences in water stable soil aggregates (WSA) between the soils which followed the order  $M > R > H > S$ , and WSA stability was significantly correlated with the root or hyphal length. The numbers of colony forming units of the microflora (total bacteria, actinomycetes, anaerobes, phosphorus solubilizers and non-AM fungi) were in general not correlated with root or hyphal length but in some cases, were significantly correlated with WSA. Bacteria isolated from the water-stable soil aggregate fraction tended to be more numerous than from the unstable fraction. The difference was significant in the M soil for total bacteria and P solubilizing bacteria. Non-AM fungi were more numerous in the unstable fraction (Andrade, Mihara, Linderman *et al.* 1998).

In further studies conducted at the above Laboratory, *Glycine max* was grown in pot cultures in unsterilized soil, fertilized with  $\text{NO}_3$  or  $\text{NH}_4$  fertilizers, and inoculated with *Bradyrhizobium japonicum*. Water soluble aggregates (WSA) correlated positively with root and AM soil mycelial development but negatively with total bacterial counts. Soil arthropods (Colembola) numbers were negatively correlated with AM hyphal lengths. Soils of nodulated and ammonia fertilized plants had the



highest level of WSA and the lowest pH in week 9. Sparse root development in the soils of N deficient control plants indicated that WSA formation was primarily influenced by AM hyphae. The ratio of bacterial counts in the water stable versus water unstable soil fraction increased for the first six weeks and then declined, while counts of anaerobic bacteria increased with increasing WSA. The number of soil invertebrates (nematodes) and protozoans did not correlate with the bacterial counts or AM soil hyphal lengths. Soil pH did not affect mycorrhiza development but actinomycetes counts declined with decreasing soil pH. AM fungi and roots interacted as the factors that affect soil aggregation, regardless of N nutrition (Bethlenfalvay, Cantrell, Mihara *et al.* 1999)

### **Mycorrhizal colonization at sites of volcanic eruptions**

Studies were conducted at the Department of Biology and Ecology, Utah State University, Logan, Utah, USA, to describe some events that might explain differential survival of some patches of *Lupinus lapidus* in the vicinity of Mount St. Helens. The studies indicated that gophers acted as vectors for the VAM fungi as spores of VAM fungi were collected from their faeces. It may have been possible that the defecation of VAM inocula by an animal from a nearby refuge in association with a grown seedling could be responsible for establishment of VAM symbiosis which may be important to the long term growth and survival of many plants (Allen and MacMohan 1988)

Studies conducted at the National Tropical Botanical Garden, Hawaii, USA, on plants colonizing 8- and 14-year-old lava flows, a 28-year-old cinder fall, a 137-year old volcanic soil, and a geothermic volcanic soil showed that VAM was present in samples collected from all sites and the frequency of occurrence and intensity of root colonization increased with the age of the site. At the youngest site, 57% of the sampled species were mycorrhizal and this increased to 84% in the cinder fall, and 100% in the old soil. Orchard and ericoid mycorrhizas were also present in some sites. Native Hawaiian species tended to form mycorrhizae more frequently and had more intense mycorrhizal infection than did alien species. Plant succession at Hawaiian volcanic sites differed somewhat from a productive model in the lack of dominance by non-mycotrophic species in the early stages. The difference apparently resulted from the arrival of mycorrhizal fungi soon after the volcanic surface cooled and the paucity of non-mycotrophic species available to invade very recent volcanic sites. (Gemma and Koske 1990)

Studies were conducted at the Department of Biology, Systems Ecology Research Groups, San Diego State University, California, USA, on three major types of disturbed habitats resulting from the

eruption of Mount St. Helens in 1980: a sterile pyroclastic flow site, a blast zone site with all of the vegetation destroyed and tephra deposited on the surface, and a high ashfall region with deposited tephra over surviving plants. The studies showed that 10 years after eruption, both ecto- and VA mycorrhizas re-established in the disturbed areas primarily through inoculation from the buried old soils, a process positively facilitated by gophers. Mycorrhizas always formed at a quicker rate in the less disturbed areas of the eruption site. Detailed observations on the sterilized pumice plain indicated that through 1990, the ectomycorrhizas were poorly developed and no fruiting structures were seen. This may be related to either slow soil development and/or compatibility restrictions. These results indicate that the magnitude of the disturbance regulates the rate of recovery not only of the vegetation, but also of both the VA and ectomycorrhizal fungi (Allen, Crisafulli, Friese *et al.* 1992).

Studies conducted at the Hokkaido University, Hokkaido, Japan on revegetation of volcanic eruptions by *Larix kaempferi* showed that 1-5 year old seedlings were infected with 12 types of ectomycorrhizas. At low and intermediate elevations, seedlings were colonized by one or two types while at higher elevations they were colonized by two or three types of mycorrhizas. Under the more stressed environments of high and intermediate elevations, the mean dry weights of one year old seedlings, 40% of which were colonized by three or four mycorrhizal fungi, were double the weights of the same aged seedlings at lower elevations where only 10% of seedlings were colonized by three or four mycorrhizal fungi. (Yang, Cha, Shibuya *et al.* 1998)

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# Research findings

## Effect of *Glomus fasciculatum* on seedlings growth of *Sorghum vulgare*

R K Aher<sup>1</sup>, L N Nair<sup>2</sup> and A A Kulkarni<sup>3</sup>

<sup>1</sup>Department of Botany, New Arts, Commerce and Science College, Parner, Dist: Ahmednagar (Maharashtra), E-mail: aher-rk@yahoo.com

### Introduction

*Sorghum vulgare*, Pers. is an important food and fodder crop in India and in Maharashtra it is the major food grain crop. This multipurpose crop has now become an industrial crop, and used to produce alcohol, beer, starch, and jaggery.

Earlier researchers have documented that the inoculation of AMF is beneficial to crops by way of mobilizing nutrients especially P (Lambert, Baker, and Cole 1980; Sekar, Vanangamudi, and Suresh 1997). The symbiotic association benefits plants by improving nutrient uptake (Mosse 1973). Recent investigations reveal that mycorrhizal fungi enhance the growth and productivity of different crops (Khaliq, Gupta, and Kumar 2001; Rajeshwari, Latha, Vanangamudi *et al.* 2001). AMF have been found to associate with plants in natural communities accounting for more than 1000 genera belonging to 200 different families, of which sorghum is one. Therefore, selection of a most suitable strain of AMF for sorghum is necessary for effective growth of seedlings. The present investigation was carried out to achieve this; this information will be useful to plant breeders.

### Materials and methods

Pot culture experiments were conducted using compatible strains of AM (*Glomus fasciculatum*) spores from the rhizospheric soil of sorghum. For raising sorghum seedlings three pots were used; the first pot was the control with sterile soil; the second and third pots were filled with sterile soil with 60 and 120 AM spores, respectively. Three replicates were used for each set. Three sterile pregerminated seeds of sorghum were placed in all the three pots and covered with a thin layer of soil. At 40 days after planting, seedlings were evaluated for number of leaves, length of shoot and root, fresh and dry weight of shoot and root, number of spores in soil and percentage infectivity in roots. Spores were extracted from the soil by the wet sieving and decanting method (Gerdemann and Nicholson 1963). AM infection percentage was also calculated (Phillips and Hayman 1970). The data were analyzed statistically.

### Results and discussion

Association of VAM fungi had a positive effect on sorghum seedlings. *Glomus fasciculatum* inoculated plants grew more luxuriantly than the control. In the first set of pots (control) the plants had very feeble growth. The length of shoot (30.0 cm) and root (6.8 cm), the fresh (16.0 g) and dry weight (4.8 g) of shoot and root (4.2 g, 0.9 g) were much lower in the controls. However, pots supplemented with 60 spores had shoots 32.7 cm long, roots 7.3 cm long, and the fresh and dry weights of shoot 18.5 g and 5.7 g and root (4.6 g and 1.0g) were more than those of the control. There was an increase in the number of VAM spores (28) and percentage infection (50%). Similar results were obtained in pots supplemented with 120 spores (Table 1). Results clearly indicate that compatible strains of AM increase the growth and biomass of

Table 1 Effect of *G. fasciculatum* on sorghum seedlings

Parameter	Plant organ	Control pot	Pot 1 with 60 spores	Pot 2 with 120 spores
Leaf number	—	12 ± 1	15 ± 1	19 ± 1
Length (cm)	Leaf	36.0 ± 2.80	32.7 ± 3.0	37.6 ± 2.88
	Shoot	30.0 ± 2.20	32.7 ± 2.8	37.5 ± 3.40
	Root	6.8 ± 0.80	7.3 ± 1.0	7.9 ± 1.0
Fresh weight (g)	Shoot	16.0 ± 1.20	18.5 ± 1.6	19.6 ± 1.48
	Root	4.2 ± 0.40	4.6 ± 0.80	5.2 ± 1.0
Dry weight (g)	Shoot	4.8 ± 0.80	5.7 ± 1.22	6.6 ± 0.82
	Root	0.9 ± 0.20	1.0 ± 0.10	1.4 ± 0.22
Spore number	—	—	28 ± 1.33	34 ± 3.12
% infectivity	—	—	50 ± 1.80	60 ± 2.10

Mean value of three replicates

seedlings. Association of *Glomus* with palmarosa and its positive influence on growth and biomass production was reported by Gupta, Janardhanan, Chatopadhyay *et al.* (1990). A similar trend was also observed in peppermint (Khaliq, Gupta, and

<sup>2</sup> Department of Botany, University of Pune, Pune-411 007

<sup>3</sup> Department of Botany, Ahmednagar College, Ahmednagar

Kumar 2001). The present investigations indicated that growth and biomass of *S. vulgare* significantly increased through inoculation of VAM spores.

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## Role of VA - mycorrhizal biofertilizer in establishing black gram (*Vigna mungo* L.) var-T9 in abandoned ash ponds of Neyveli Thermal Power Plant

A Merline Sheela and M D Sundaram

Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu

### Introduction

During the last few decades, there has been a dramatic increase in coal ash production worldwide due to increased amounts of electricity being generated by coal fired power plants. Coal and lignite in particular comprise about 75 per cent of the world resources of fossil fuels. A number of researchers (Clarke 1993; Manz 1993; Manz 1998) have compiled extensive data on the production and utilization of coal fly ash. Fly ash is mostly dumped wet into ash ponds. It creates environmental hazards and also occupies vast tracts of useful land. Fly ash contains several toxic heavy metals, contaminates ground water, and is a health hazard (Adholeya 2000). Studies show that with biological intervention one can grow commercially important plant species on fly ash covered areas (Adholeya

2000). Mycorrhizal association benefits higher plants by improving water and nutrient uptake, helping in the development of roots and binding the soil, storing carbohydrates and oil, protecting plants from soil-borne diseases, and detoxifying soils contaminated by metals.

Ash ponds are found to have no significant microbial activity. The present study was undertaken to introduce and study the role of the root associated VA mycorrhizal 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.

### Materials and method

A field experiment was laid out in Randomized Block Design (RBD) in the ash ponds.

*Physical properties of pond ash*

Bulk density : 1.44  
Specific gravity : 2.35

*Chemical properties of pond ash*

Silica, SiO<sub>2</sub> (%) : 73–80  
Alumina, Al<sub>2</sub>O<sub>3</sub> (%) : <1.00  
Iron, Fe<sub>2</sub>O<sub>3</sub> (%) : 10–16  
pH (1:2.5) : 3.0–8.0  
EC (mmho/cm) : 0.3–1.7  
Organic carbon (%) : 0.2–0.3  
Nitrogen (kg/ha) : 50–75  
Phosphorus (kg/ha) : 3.0–8.0  
Potassium (kg/ha) : 50–60

The pond ash was enriched with the following organic materials.

Red earth @ 75 T/ha  
Farm yard manure @ 37.5 T/ha  
Lignite fly ash @ 50 T/ha  
Coir pith @ 75 T/ha

All these amendments were incorporated basally, three weeks prior to sowing.

The following treatments were given.

- T1 - Uninoculated control
- T2 - *Glomus mosseae* alone
- T3 - *Rhizobium* sp. AU-1 (local isolate)
- T4 - *Rhizobium* sp. PAB-1 (stress tolerant isolate)
- T5 - *Glomus mosseae* + *Rhizobium* sp. AU-1
- T6 - *Glomus mosseae* + *Rhizobium* sp. PAB-1

Parameters such as plant height, biomass production, VAM colonization, and yield were studied. The grains were analysed for nitrogen, phosphorus, heavy metal, and micronutrient content.

The VAM species, *Glomus mosseae*, was maintained on onion (*Allium cepa* L.) to prepare pot cultures following the procedure of Krishna, Shetty, Dart *et al.* (1985). Once the pot culture was established, its soil along with the roots of onion was used as a source of inoculum. The inoculum was used @10 kg/ha in the ash pond. The rhizobial isolates were grown in yeast extract

mannitol agar medium for mass multiplication and then mixed with lignite to prepare the carrier based inoculum. The lignite based rhizobium inoculum @4 kg/ha containing 10<sup>10</sup> cells of rhizobium per gram of inoculum was used to treat the black gram seeds.

## Results and discussion

Analysis of abandoned ash of Neyveli Thermal Power Plant showed a pH that varied from 3.0 to 8.0, silica levels between 75% and 80%, iron from 10%–16%, and a poor N, P, and K content. The crop associative microbes viz. VAM fungi and *Rhizobium* were not encountered.

In the present study, best effects were noticed in plants inoculated with VAM fungus *Glomus mosseae* and *Rhizobium* sp. PAB-1. The plant height, dry weight, and pod yield increased when *G. mosseae* was inoculated along with *Rhizobium* sp. PAB-1 (yield 2568 kg/ha) (Table 1). The enrichment of pond ash with organic materials enhanced its capacity to retain moisture and support plant growth. Also on decomposition, organic material produce acids that combine with some heavy metals to form compounds that are less mobile and therefore less likely to pollute ground water and surface runoff. It is possible that VAM infection increases the capacity of plants to accumulate phosphate as it is released by other microorganisms.

The VA mycorrhizal colonization was also greater (88%) when *G. mosseae* was inoculated along with *Rhizobium* sp. PAB-1. The nitrogen and phosphorus content of the grains was also increased (Table 2). Results showed that in general plants did not accumulate toxic heavy metals (Table 3). Also the micronutrient uptake increased in VA mycorrhiza inoculated plants. It is clear that mycorrhizal association improves the water and nutrient uptake by plants, helps root development by binding the soil and detoxifying heavy metals.

The pond ash is poor in nutrients, but it contains toxic heavy metals and essential nutrients such as phosphorus, calcium, and magnesium. The VAM helps in binding the fine particles of ash

**Table 1** Effect of inoculation of VAM and Rhizobium on growth, dry weight, and pod weight of black gram (*Vigna mungo* L.) in ash pond

Treatment	Plant height (cm)		Dry weight (g)		
	25 DAS	50 DAS	50 DAS	At harvest	Pod yield kg/ha
Uninoculated control	14.5	29.7	4.50	10.00	812
<i>Glomus mosseae</i>	15.7	32.9	1.86	13.20	1188
<i>Rhizobium</i> sp. AU-1	16.1	35.6	2.22	17.34	1762
<i>Rhizobium</i> sp. PAB-1	18.8	38.2	3.60	21.12	2059
<i>Glomus mosseae</i> + <i>Rhizobium</i> sp. AU-1	20.0	41.1	5.62	22.92	2297
<i>Glomus mosseae</i> + <i>Rhizobium</i> sp. PAB-1	21.7	46.3	59.0	7.94	2568
CD (P = 0.5%)	2.1	2.0	0.31	1.70	385.72

\* values represent average of three determinations

**Table 2** Effect of inoculation of *G. mosseae* and *Rhizobium* in VAM-colonization, nitrogen and phosphorus contents of black gram (*Vigna mungo* L.) var T-9 in ash pond

Treatments	VAM colonization*			Nitrogen content %*			Phosphorous content %*		
	25 DAS	50 DAS	At harvest	25 DAS	50 DAS	At harvest	25 DAS	50 DAS	At harvest
Uninoculated control	8	15	10	0.84	1.85	2.65	0.04	0.06	0.08
<i>Glomus mosseae</i>	53	65	45	0.88	1.88	3.37	0.15	0.21	0.32
<i>Rhizobium</i> sp. AU-1	10	20	12	0.91	1.93	4.75	0.06	0.11	0.14
<i>Rhizobium</i> sp. PAB-1	12	23	10	0.09	1.98	4.82	0.08	0.18	0.22
<i>Glomus mosseae</i> ± <i>Rhizobium</i> sp. AU-1	60	84	50	0.98	2.05	4.91	0.22	0.31	0.38
<i>Glomus mosseae</i> ± <i>Rhizobium</i> sp. PAB-1	63	88	55	1.15	2.87	4.98	0.28	0.38	0.41

\* values represent average of 3 determinations

**Table 3** Micronutrient and heavy metal content in the grains of black gram (*Vigna mungo* L.) var-T9 inoculated with *G. mosseae* and *Rhizobium* PAB-1 in ash pond

Metals	Uninoculated* ( $\mu\text{g/g}$ )	Inoculated* ( $\mu\text{g/g}$ )
Zinc (Zn)	23.40	28.54
Iron (Fe)	51.53	69.75
Manganese (Mn)	32.40	36.30
Copper (Cu)	13.70	16.10
Cobalt (Co)	0.02	0.023
Chromium (Cr)	0.71	0.65
Cadmium (Cd)	0.08	0.05
Lead (Pb)	BDL	BDL
Nickel (Ni)	2.20	2.13

\* Values represent average of 3 determinations  
BDL - below detectable limit

and arrests the movement of heavy metals in the ash ponds. It also helps in the uptake of micronutrients and phosphorus mobilization. Plants such as marigold, tuberose, carnations, and sunflower have been cultivated successfully in fly ash ponds with VA mycorrhizal inoculation (Adholeya 2000). Better yield in cucumber was obtained in ash ponds on incorporation of various organic amendments (Manivannan and Baskaran 2001).

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; Adriano, Woodford, and Ciravalo 1980) and used to correct nutrient deficiency or increase nutrient uptake of crops (Martens 1970).

Arbuscular mycorrhiza benefit plants by improving the supply of nutrients, especially phospho-

rus and minerals such as zinc, copper, sulphur, potassium, and calcium (Cooper and Tinker 1978), and the plant supplies the fungus with photosynthetic sugars (Verma and Schuepp 1995), increased tolerance of mycorrhizal plants to toxic heavy metals concentrations in the soil makes mycorrhiza significant. Mycorrhizal fungi and organic matter can be very well used in an inert medium like pond ash for growing crop plants.

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## Effect of cropping sequence on colonization and population of VA-mycorrhiza and root-knot nematode and yield of urdbean (*Phaseolus mungo* Roxb.)

A Hasan<sup>1</sup>, M Naeem Khan<sup>2</sup> and M Nehal Khan<sup>2</sup>

Department of Nematology<sup>1</sup> and Pathology<sup>2</sup>, N D University of Agriculture and Technology, Kumarganj, Faizabad – 224 229, India

### Introduction

Urdbean (*Phaseolus mungo* Roxb.) is one of the most important pulses in India. It is generally grown on marginal lands with low fertilizer inputs like other pulses. It is primarily a kharif crop but is grown now as a summer crop also where irrigation is possible. It is often infected by root-knot nematode (*Meloidogyne* spp.) which causes 23%–49% yield losses (Ali 1997). VA-mycorrhizae have long been known to enhance the growth/yield of various crops (Gerdemann 1968; Smith and Read 1997). Pulses are highly dependent on mycorrhizae due to their high P requirement (Bethlenfalvay 1993). The density of their spores in the field and/or the degree of root colonization are considered measures of plant performance (Fyson and Oaks 1990; Wani, McGill, and Tiwari 1991) as most of the plants are mycorrhizal in nature (Gerdemann 1968). Besides other factors, crop management practices such as fallowing, and rotations involving non-hosts to mycorrhizae (Bagyaraj 1994; Black and Tinker 1979; Thompson 1987; Wani and Lee 1995) have been found to adversely affect the spore density and/or root colonization. These management practices influence the nematode population also (Johnson 1985; Swarup 1995). Besides these man-

agement practices, VAM fungi have also been reported to suppress various nematodes including root-knot (*Meloidogyne* spp.) in agricultural crops (Hussey and Roncadori 1982; Francl 1993).

In view of the above, a field experiment was laid to find out the crop sequence suitable for cultivation of *zaid urdbean* in a field infested with VAM fungus, *G. mosseae* and root-knot nematode, *M. incognita*.

### Materials and methods

Seven crops sequences (Table 1) were tested in naturally infested 10 x 4 m<sup>2</sup> plots infested with VAM fungus, *Glomus mosseae* and root-knot nematode, *Meloidogyne incognita*. The initial population of VAM fungus and the root-knot nematode was determined before setting up the experiment. The experiment was carried out using the Randomized Block design with three replications of each treatment (crop sequence). Appropriate crop protection measures against the insect were applied. VAM spore density, root colonization, nematode density, and root galls on *kharif* as well as *zaid urdbean* were recorded at the flowering stage of the crop. The VAM spores were quantified following the wet



**Table 1** Crop sequences vis-à-vis season.

Sequence	Season		
	<i>Kharif</i>	<i>Rabi</i>	<i>Zaid</i>
1	Maize	Potato	Urdbean
2	Maize	Mustard	Urdbean
3	Paddy	Mustard	Urdbean
4	Paddy	Wheat	Urdbean
5	Urdbean	Mustard	Urdbean
6	Urdbean	Potato	Urdbean
7	Urdbean	Peas	Urdbean

Maize = *Zea mays*, Mustard = *Brassica nigra*, Paddy = *Oryza sativa*, Peas = *Pisum sativum*, Potato = *Solanum tuberosum*, Wheat = *Triticum aestivum*

sieving and decanting technique of Gerdemann and Nicholson (1963) and the mycorrhizal root colonization by clearing the roots in near boiling 10% KOH aqueous solution for 48 hours and then staining in trypanblue following several washings in distilled water to drain out KOH (Phillips and Hayman 1970a). Stained roots were cut into 1 cm segments and 50–100 such segments were examined under a microscope. The mycorrhizal colonization was determined by Nicholson's formula (1955) as follows:

$$\text{Percent colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

The nematode population in soil was determined following Cobb's sieving and decanting method coupled with Baermann's funnel techniques (Flegg and Hooper 1970). The incidence of nematode diseases was recorded as measure of gall index on a scale of 0–4 (0 = no gall formation, 1 = 25%, 2 = 50%, 3 = 75% and 4 = 100% of roots galled). Yield was also recorded at harvest. Data were subjected to analysis of variance and treatments were compared using Duncan's multiple range test (Steel and Torrie 1980). Nematode and spore count data were transformed to log (X+1) before analyzing them (Proctor and Marks 1974).

## Results

It is evident from Table 2 that 90%–95% of the roots were colonized by the VAM fungus during *kharif* and 35%–77% during *zaid*. It was significantly low (35%–45%) under maize-mustard-urdbean, paddy-mustard-urdbean and urdbean-mustard-urdbean crop sequences during *zaid*. The VAM spore population around *kharif* urdbean increased by 2.4–2.5 times compared to the initial population whereas it decreased by

88.6%–91.0% during *zaid* under maize-mustard-urdbean, paddy-mustard-urdbean and urdbean-mustard-urdbean. The spore density under maize-potato-urdbean, paddy-wheat-urdbean, urdbean-potato-urdbean and urdbean-peas-urdbean fluctuated around the initial population (15886 spores/kg soil).

**Table 2** Effect of cropping sequence on mycorrhizal root colonization and soil spore population of VAM fungus, *Glomus mosseae* around urdbean during *kharif* and *zaid* seasons (Initial VAM population - 15886 spores/kg soil)

Treatment			Crop season	
Crop sequence vis-à-vis season			<i>Kharif</i>	<i>Zaid</i>
<i>Kharif</i>	<i>Rabi</i>	<i>Zaid</i>	Mycorrhizal colonization (percentage)	
Maize-	Potato	Urdbean	–	71a
Maize-	Mustard-	Urdbean	–	35b
Paddy-	Mustard-	Urdbean	–	41b
Paddy-	Wheat-	Urdbean	–	75a
Urdbean-	Mustard-	Urdbean	95a	45b
Urdbean-	Peas	Urdbean	90a	75a
Urdbean-	Peas-	Urdbean	92a	77a
VAM spore population/ kg soil				
Maize-	Potato-	Urdbean	–	15733a
Maize-	Mustard-	Urdbean	–	1817b
Paddy-	Mustard-	Urdbean	–	1527b
Paddy-	Wheat-	Urdbean	–	16833a
Urdbean-	Mustard-	Urdbean	38033a	1430b
Urdbean-	Potato-	Urdbean	38466a	15666a
Urdbean-	Peas-	Urdbean	39816a	15200a

Figures followed by similar letters do not differ significantly (P = 0.05)

The root-knot disease in *kharif* urdbean recorded in terms of the gall index on a scale of 0–4 ranged from 3.8–3.9 under urdbean-mustard-urdbean, urdbean-potato-urdbean and urdbean-peas-urdbean whereas it developed to a very low extent (1.1–1.2) under all the crop sequences barring maize-potato-urdbean, urdbean-potato-urdbean and urdbean-peas-urdbean (Table 3). The nematode population around *kharif* urdbean increased by 4.9–5.7 times the initial population (600/kg soil) while it decreased by 79.7%–84.2% (of the initial population) during *zaid* under maize-mustard-urdbean, paddy mustard-urdbean, paddy-wheat-urdbean and urdbean-mustard-urdbean. The population under maize-potato-urdbean, urdbean-potato-urdbean and urdbean-peas-urdbean fluctuated around the initial population.

The yield of *kharif* and *zaid* urdbean ranged from 765–780 and 565–680 kg/ha, respectively

**Table 3** Effect of cropping sequence on root gall development and soil population of root-knot nematode, *Meloidogyne incognita* around urdbean during *kharif* and *zaid* seasons

Treatment			Crop season	
Crop sequence vis-à-vis season			<i>Kharif</i>	<i>Zaid</i>
<i>Kharif</i>	<i>Rabi</i>	<i>Zaid</i>	Root Gall Index (0–4 scale)	
Maize-	Potato-	Urdbean	–	2.4a
Maize-	Mustard-	Urdbean	–	1.2b
Paddy-	Mustard-	Urdbean	–	1.1b
Paddy-	Wheat-	Urdbean	–	1.2b
Urdbean-	Mustard-	Urdbean	3.9a	1.2b
Urdbean-	Potato-	Urdbean	3.8a	3.1c
Urdbean-	Peas-	Urdbean	3.8a	2.0d
Nematode population/ kg soil				
Maize-	Potato-	Urdbean	–	625a
Maize-	Mustard-	Urdbean	–	113b
Paddy-	Mustard-	Urdbean	–	95b
Paddy-	Wheat	Urdbean	–	122b
Urdbean-	Mustard-	Urdbean	324a	102b
Urdbean-	Potato-	Urdbean	3423a	885c
Urdbean-	Peas-	Urdbean	3021a	552a

Figures followed by similar letters do not differ significantly (P=0.05)

(Table 4). It was significantly lower under maize-mustard-urdbean, paddy-mustard-urdbean and urdbean-mustard-urdbean compared to the remaining four sequences tested during *zaid*. The highest yield (680 kg/ha) recorded was in the paddy-wheat-urdbean sequence.

**Table 4** Effect of cropping sequence on yield of urdbean in field infested with VAM fungus, *Glomus mosseae* and root-knot nematode, *Meloidogyne incognita* during *kharif* and *zaid* seasons

Treatment	Crop season	
	<i>Kharif</i>	<i>Zaid</i>
Crop sequence vis-a vis season kharif-rabi-zaid	Yield (kg/ha)	
Maize-Potato-Urdbean	-	625a
Maize-Mustard-Urdbean	-	565b
Paddy-Mustard-Urdbean	-	570b
Paddy-Wheat-Urdbean	-	680c
Urdbean-Mustard-Urdbean	770a	575b
Urdbean-Potato-Urdbean	765a	630a
Urdbean-Peas-Urdbean	780a	618a

Figures followed by similar letters do not differ significantly (P = 0.05)

## Discussion

The reduction in nematode population, root galls, mycorrhization, spore density and yield of *zaid* urdbean under various crop sequences vis-a-vis *kharif* urdbean could not only be attributed to the hot weather conditions prevailing during *zaid* alone but also to mustard and/or wheat crops which preceded it. Mustard is known as non-mycorrhizal in nature (Baltruschat and Dehne 1989; Black and Tinker 1979; Smith and Read 1997) possessing nematocidal properties in its root exudates (Hasan 1992) and wheat as non-host to root-knot nematode, *M. incognita* (Saka and Carter 1987). Thus, these crops might have led to a drastic reduction of VAM spores (mustard only) and root-knot nematode population (both mustard and wheat) with the consequence that mycorrhization and nematode infection of the subsequent urdbean remained low despite the fact that this crop is a good host to VAM mycorrhiza and root-knot nematodes. Out of seven crop sequences tested in VAM and root-knot nematode infested fields, the paddy-wheat-urdbean sequence was found to be the best one as the highest yield of 680 kg/ha was recorded for *zaid* urdbean. This could have been the reason for the popularity of this sequence among the farmers.

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# Distribution of AM fungi along salinity gradient in a salt pan habitat

R Abdi and H C Dube

Department of Life Sciences, Bhavnagar University, Bhavnagar -364 002, Gujarat, India  
E-mail: rupaabdi@hotmail.com

## Introduction

Inland salt pans offer an opportunity to study the effects of soil salinity on AM fungal-halophyte associations without the confounding influence of tidal inundations. Soil salinity exerts powerful effects on both, the distribution of individual plant species and community structures (Ungar 1996). Salinity can also affect the distribution and abundance of mycorrhizal fungi. Soil salinity gradients, despite being common in coastal and inland ecosystems, have rarely been the focus of studies of mycorrhizal fungi (Johnson-Green, Kinkel, and Booth 1995).

The Bhal, near Bhavnagar, is a low lying area, which periodically gets inundated by the back waters of the Gulf of Khambhat. As a result, it suffers from chronic soil salinity. Solar salt harvesting is one of the major economic activities in this area. Soil salinity (sodium content) in these salt pans can go up to more than 75000 mg/g. The only plant that survives such extreme salinity is *Suaeda nudiflora*, which grows in and around the periphery of these salt pans.

## Materials and methods

To ascertain the effect of salinity gradient on arbuscular mycorrhizal association in *Suaeda nudiflora*, a transect was run from the centre of a salt pan area to its outer edge. Samples of rhizosphere soils (0–20 cm deep), and young primary roots, were collected at approximately 1 metre distance, up to nine metres from the periphery towards the centre of the salt pan. Five different plants, at each site, were selected for sampling. Samples of rhizosphere soils were taken at a standard distance from the stem (depending on the size of the plant), while samples of primary roots were taken at random from 0–20 cm depth. Five sets of root and rhizosphere samples, per site, were thoroughly mixed and a sub-sample was taken for analysis of colonization. Arbuscular mycorrhizal spores were extracted from the soil by wet sieving and decanting methods of Gerdemann and Nicholson (1963) and sucrose centrifugation, as given by Brundrett, Melville, and Peterson (1994). Arbuscular mycorrhizal fungi were identified using the keys of Morton and Benny (1990), Schenck and Perez (1990) and Mehrotra and Bajjal (1994). Spore density (SD) was measured by counting the spores per 100 g of rhizosphere soil; species richness (SR) represented the number of AM fungal species present in the soil samples. Roots were cleared and stained by the method of Phillips and

Hayman (1970), as modified by Brundrett, Melville, and Peterson (1994). For estimation of percent root colonization (PCR) the gridline intersection method of Giovannetti and Mosse (1980) was used. Soil pH was determined by a digital pH meter (Elico L1 120). Soil moisture was measured following the method given by Trivedi, Goel and Trisal (1987). Soil salinity was measured as electrical conductivity by the method given by Jackson (1973).

## Results and discussion

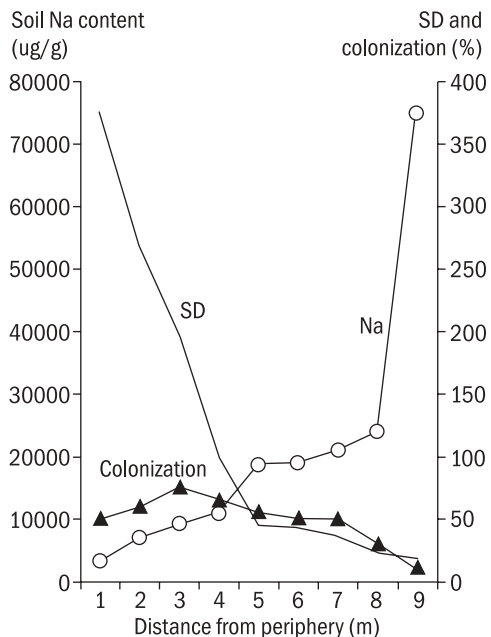
The data (Table 1 and Figure 1), suggest that the sodium content increased from the periphery towards the centre of the salt pan. The increase was initially gradual and quite abrupt later. The increase in sodium content, from 3270–75000 µg/g, of the soil from the periphery to the centre of the salt pan, was accompanied by a decrease in fungal features, such as percent root colonization, spore density, and species richness.

The percent root colonization (Table 1), after an initial rise, declined from 50% to 10%. The spore density declined from 376 to 18 and so did the species richness, which declined from 6 to 1. With the rise in sodium content, there was a gradual decline in percent root colonization (Figure 1) but a sharp decline in spore density and species richness (Figure 2).

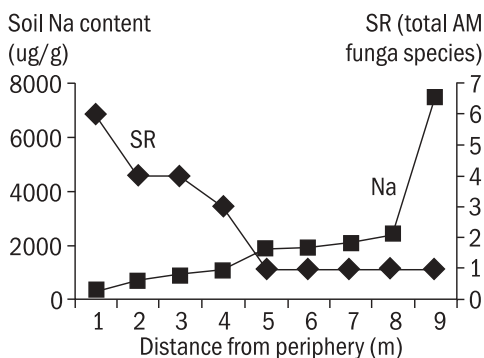
There was a strong negative correlation between soil sodium and percent root colonization ( $r = -0.908$ , critical value being  $\pm 0.632$  at  $p = 0.05$ ); colonization was significantly negatively correlated

**Table 1** Arbuscular mycorrhizal fungal and rhizosphere soil characteristics of *Suaeda nudiflora* along a salinity gradient in a salt pan, from the periphery towards the centre (m: metres from periphery)

Soil site (m)	Soil Na (mg/g)	Coloni- zation (%)	SD (spores/ 100 g)	Total AM species	Soil pH	Soil moisture (%)
1	3270	50	376	6	8.7	45
2	6971	60	270	4	8.3	40
3	9110	75	198	4	8.2	38
4	10971	65	100	3	8.4	40
5	18701	55	45	1	8.1	43
6	19000	50	42	1	8.3	44
7	20940	50	36	1	8.2	43
8	24000	30	23	1	8.7	43
9	75000	10	18	1	8.6	45



**Figure 1** Correlation between soil sodium (Na), percent root colonization and spore density (SD)



**Figure 2** Correlation between soil sodium (Na) and AM fungal species richness (SR)

to species richness ( $r = -0.635$ ), and weakly negatively correlated to spore density ( $r = -0.620$ ). This implied that the AM fungal colonization was relatively more vulnerable to soil salinity than the fungal spores, probably because the thick walls and peridium of the spores (in case of *Glomus* sp. 18RA) imparted tolerance to high salinity. Secondly, very few species (one or two) of AM fungi could survive under the highly saline environment, and this resulted in a sharp decline in species richness.

The spore density of AM fungal species present in the rhizosphere along the increasing salinity gradient, is presented in Table 2. Only one genus, *Glomus*, was present in the saltpan habitat and *Glomus* sp.18RA was the lone species which was present at all the sites: 1 to 9 metres from the periphery to centre, thus withstanding high salinity of over 18 000  $\mu\text{g/g}$  at the 5 metre site to up to 75 000  $\mu\text{g/g}$  sodium at Site 9. *Glomus microaggregatum* and

**Table 2** Spores density (spores/100 g of soil) of various AM fungi at increasing salinity gradients from periphery towards centre.

AM fungi	SITES								
	Distance from periphery to centre of salt pan (in m)								
	1	2	3	4	5	6	7	8	9
<i>Glomus</i> sp.18RA	200	150	98	50	45	42	36	23	18
<i>G. microaggregatum</i>	50	42	34	30	0	0	0	0	0
<i>G. sp. 12RA</i>	36	40	33	20	0	0	0	0	0
<i>G. sp. 3RA</i>	32	38	33	0	0	0	0	0	0
<i>G. sp.14RA</i>	30	0	0	0	0	0	0	0	0
<i>G. sp. 16RA</i>	20	0	0	0	0	0	0	0	0

*Glomus* sp.12RA could withstand salinity up to the 4-metre site, followed by *Glomus* sp.3RA up to Site 3. The remaining two species could not survive salinity present at Site 2 onwards. The spore density values of *Glomus* sp.18RA were fourfold to twofold higher than other *Glomus* species, at all the salinity levels (Table 2).

The present study provided somewhat unexpected results indicating that plants of a typically non-mycorrhizal family (*Chenopodiaceae* in this case) could be colonized by AM fungi, which is partly in agreement with the literature (Hildebrandt, Janetta, Ouziad, *et al.* 2001; Johnson-Green, Kinkel, and Booth 1995; Kim and Weber 1985; Pond, Menge, and Jarrell 1984; Sengupta and Chaudhari 1990; Van Duin, Rozema, and Ernst 1989).

While we have recorded the presence of six species (belonging to one genus) of AM fungi in the salt pan habitat, Pond, Menge, and Jarrell (1984) found six species (spread over three genera), and Hildebrandt, Janetta, Ouziad *et al.* (2001) reported only one species, *Glomus geosporum*, as the dominant AM fungi in a Central European salt marsh environment.

Our observation of colonization in extremely saline soils (75 000  $\mu\text{g/g}$  sodium) is similar to the studies of Pond, Menge, and Jarrell (1984), who reported AM colonization in highly saline soils (>60 000  $\mu\text{g/g}$  sodium). However, none of the later studies have reported mycorrhizal activity under such highly saline environments. Kim and Weber (1985) and Johnson-Green, Kinkel, and Booth (1995) found AM fungal activity to be common only in soils with sodium content less than 5000  $\mu\text{g/g}$  while Hildebrandt, Janetta, Ouziad *et al.* (2001) found up to 36% colonization and 60–90 spores/100 g in salt marsh soils with an EC of 25.8 dS/m (15 664  $\mu\text{g/g}$ ). We have found a higher level of mycorrhizal activity (percent root colonization: 50; spore density: 42) at a much higher level of salinity (19 000  $\mu\text{g/g}$ ).

*Suaeda nudiflora* was earlier reported to be non-mycorrhizal (Khan 1974; Ungar 1991), let alone

such a high degree of colonization. This perennial plant colonizes saltpan habitats with a salt concentration that no other plant could tolerate.

The symbiosis between *Suaeda nudiflora* and *Glomus* sp18RA could be due to their co-evolution in these highly saline habitats. It is possible that the fungal structures bind or exclude sodium chloride and may thereby impart salinity tolerance to the plants in these habitats, as recently described for heavy metals (Hildebrandt, Janetta, Ouziad et al 2001). These inland areas suffer from droughts over long periods during the summer months. This halophyte may have to, thus, endure a 'physiological drought' (Schimper 1898) which may be partly alleviated by the effective water and nutrient exploitation of the soils by the mycorrhizal hyphae.

All the available data indicate that mycorrhizae do play a vital role in such extreme habitats, and have a great potential in reclamation of saline wastelands.

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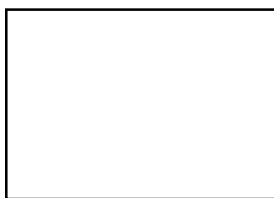
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*Agriculture, Ecosystems and Environment* **29**: 107–110

## New approaches

### Combined morphological and molecular approach to AM identification

Morphological features of resting spores and information from nucleotide sequences of ribosomal RNA were used by Lanfranco L, Bianciotto V, Lumini E, Souza M, Morton J B, Bonfante P (2001) to characterize seven mycorrhizal fungal isolates in *Gigaspora* from different geographical areas (New Phytologist 152(1): 169–179). Detailed observations were made under the light microscope on single spores mounted in Melzer's reagent and a polyvinyl alcohol-lactic acid-glycerol medium to resolve size, colour, and cell wall structures. Neighbour-joining analyses were carried out on a portion of the 18S gene and on the internal transcribed spacer (ITS) region amplified by PCR from

multisporal DNA preparations. The combined data allowed the design of oligonucleotides that unambiguously distinguished *G. rosea* from *G. margarita* and *G. gigantea* and also identified two isolates as *G. rosea* that had been previously diagnosed as *G. margarita*. ITS sequences revealed substantial genetic variability within clones of a single isolate of *G. rosea* as well as among geographically disjunct *G. rosea* isolates. The results showed that morphological and molecular data can clarify relationships among species of low morphological divergence. Sequence information allowed the extent of genetic divergence within these species to be investigated and provided useful PCR primers for detection and identification.



### Centre for Mycorrhizal Culture Collection

### Screening of potential arbuscular mycorrhizal fungal (AMF) isolates for wheat

Reena Singh and Alok Adholeya

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

In terms of production, wheat (*Triticum* spp.) occupies the prime position among the world's food crops. In India, it is the second most important food crop, after rice and contributes about 25% to the country's total foodgrain production. Considerable scientific efforts are aimed globally towards yield improvement and, more recently, yield sustainability. The potential for wheat yields to be improved under low-input agriculture by management of mycorrhizal symbiosis is therefore of considerable importance. At the Centre for Mycorrhizal Research, TERI, a greenhouse study was conducted to test the responsiveness and efficacy of 17 AMF isolates on wheat.

Plastic pots were used to study the effect of AMF isolates from five different regions. All the isolates were collected from wheat-rice rotation fields. In the Ghaziabad region, three different fields differing in agricultural practices were selected: (1) ConT field, where pre-seeding tillage was done using tractors; (2) ZT field, where no tillage was done; (3) RBP field, where plantation was done on raised beds and no tillage was done. In the Badshahpur and Gual Pahari regions, different doses of manure and fertilizer were applied to

the wheat crop at both the sites:

- F1 : Nitrogen 100 kg/ha; phosphorus 50 kg/ha; potassium 40 kg/ha and FYM 20 tonne/ha (recommended level)
- F2 : Nitrogen 100 kg/ha; phosphorus 25 kg/ha; potassium 40 kg/ha and FYM 20 tonne/ha
- F3 : Nitrogen 100 kg/ha; phosphorus 50 kg/ha; potassium 40 kg/ha and FYM 40 tonne/ha
- F4 : Nitrogen 200 kg/ha; phosphorus 100 kg/ha; potassium 80 kg/ha and FYM 20 tonne/ha

In Budaun region three fields (LL1, low-input low yielding; LL2, low-input high yielding and HH, high input high yielding) of the wheat-rice production system were selected. In the LL1 field (low input, low yield), phosphatic fertilizers had not been applied for 4–5 years and the yield was very low. In the LL2 field, there was no application of phosphatic fertilizers and yet the field had yields comparable to those of the HH field (high input high yield), where the recommended dose of fertilizers had been applied (i.e. nitrogen 100 kg/ha; phosphorus 50 kg/ha; potassium 40 kg/ha and FYM 20 tonne/ha). Wheat had been cultivated for more than 20–25 years in all the fields selected. In the Palwal region, only organic manures

(poultry manure and FYM) were added in the field. In fertility level 1, no manure was added. In fertility levels 2 and 3, FYM was applied at the rate of 15 kg/ha and 30 kg/ha, respectively.

The AMF isolates were collected from all the selected fields (described above) and were propagated and multiplied in a greenhouse for three trap culture cycles. The experiment consisted of 18 treatments involving 17 AMF isolates (obtained from trap cultures) and one uninoculated control. The pots were arranged in a completely randomized design with four replicates per treatment. The plants were watered to a moisture level of approximately 60% of the water-holding capacity and were grown for four months in a greenhouse at  $25 \pm 5$  °C. Half-strength Hoagland's nutrient solution (Hoagland and Arnon 1938) was provided to the plants every fortnight. After four months of sowing, the crop was harvested and growth parameters viz., shoot height, dry weight, and nutrient uptake (nitrogen, phosphorus, potassium and organic carbon) recorded. The mycorrhizal parameters (AMF spore count, intraradical root colonization, infectivity potential, extramatrical hyphal biomass, succinate dehydrogenase, alkaline and acid phosphatase enzyme activity) were also analyzed.

All the samples were found to contain AMF spores. Spore number ranged from 10 to 100 per gram of soil and varied among isolates of a region. The maximum number was found in the samples inoculated with RBP isolate (i.e., the isolate collected from the field where wheat-rice plantations were grown on raised beds). All plant samples analysed for AMF colonization contained either arbuscules or vesicles or both and sometimes spores. The level of colonization in different regions ranged from 20%–60% and was found to vary significantly among isolates of the same region. No significant difference in hyphal lengths was observed amongst different isolates. However, alkaline phosphatase activity showed differences. The RBP isolate had the maximum activity. Inoculated plants had a significantly higher NPK content than their non-mycorrhizal counterparts.

In the present study, no relationship was observed between the degree of root colonization and degree of growth benefit from the symbiosis. Thus, although root colonization is obviously a necessary precursor to plant benefit from the symbiosis, in wheat the degree of benefit is not directly related to the degree of colonization. Although all isolates were able to colonize wheat and produced spores, two isolates, RBP followed by ZT, when inoculated, produced the maximum dry weight and NPK content in the shoots. These isolates also produced the maximum external hyphae and exhibited the highest alkaline phosphatase activity. This study thus, suggests that extraradical hyphae rather than spore count and root colonization should be used as indicators of the functional efficiency of AMF.

The present study supports observations from

previous studies that wheat exhibits a wide range of responses to mycorrhizal fungi (Hetrick, Wilson, and Cox, 1992, 1993; Azcón and Ocampo 1981; Kapulnik and Kushnir 1991). While previous studies (Hetrick, Wilson, and Cox 1992, 1993; Hetrick, Wilson and Todd 1996) suggest that responses to the symbiosis are consistent within a cultivar and influenced only to a limited extent by the fungal symbiont, our data suggest that mycorrhizal responses are also influenced by the fungal symbiont.

The effectiveness of the symbiosis with respect to plant growth and yield is influenced by the magnitude of the flux of phosphate (or other nutrients) to the plant and of carbohydrate to the fungus. The isolates were collected from fields where wheat-rice was cultivated. Under the wet system of rice cultivation, the land is ploughed thoroughly and flooded with 3–5 cm of standing water thus making conditions anaerobic for microbes. Wet soils normally inhibit AM formation because of poor aeration. There are reports of rare spores in permanently water-logged conditions (Khan 1974; Ragupathy and Mahadevan 1993). However, when rice is planted on raised beds, such stress is overcome and seems to favour not only AMF propagation and infectivity but also its efficacy. The isolate from the zero-tilled field was more efficient than the one from a conventionally-tilled field. Disruption to the external hyphal network of the AMF was the factor responsible for reduced P-uptake in disturbed soil (Miller 2000). Thus, the maintenance of an intact hyphal network in no-tillage systems provides both a source of inoculum of high potential, and a means of nutrient acquisition early in the growth of crops planted subsequently.

Isolates collected from the fields where higher doses of inorganic fertilizers were applied were also found to be efficient. This suggests two things: first, the applied fertilizer doses were not high enough (especially phosphorus) to inhibit AMF formation and secondly, fertilizers create a selection pressure on AMF communities and select those AMF communities that are the best mutualists. Although high-input systems are generally known to inhibit AMF formation and their functionality, there are examples of highly effective inocula obtained from conventional systems, and conversely, relatively ineffective AMF inocula were obtained from organic systems (Eason, Scullion, and Scott 1999). Thus, it will be important to improve our understanding of the processes that critically affect AMF populations under intensive management, and in particular, their recovery to full effectiveness.

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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.

- *Agriculture Ecosystems & Environment*
- *Applied Soil Ecology*
- *Aquatic Botany*
- *Biology and Fertility of Soils*
- *Brazilian Journal of Microbiology*
- *FEMS Microbiology Letters*
- *Grana*
- *Hort Technology*
- *Journal of Plant Physiology*
- *Molecular Plant - Microbe Interactions*
- *Oecologia*
- *Philippine Agricultural Scientist*
- *Plant and Soil*
- *Plant Biology*
- *Plant Journal*
- *Scientia Horticulturae*
- *Soil Biology & Biochemistry*
- *Symbiosis*

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

Name of the author(s) and year of publication	Title of the article, name of the journal, volume no., issue no., page nos [*address of the corresponding author]
YanoMelo A M, Saggin O J, and Maia L C *. 2003	<b>Tolerance of mycorrhizal banana (<i>Musa sp cv. Pacovan</i>) plantlets to saline stress</b> <i>Agriculture Ecosystems and Environment</i> 95(1): 343–348 [*Maia L C, Universidade Federae de Pernambuco, Centro de Ciências Biológicas, Department of Micologa, BR-50670420 Recife, PE, Brazil]
Gryndler M, Vosatka M*, Hrselova H, Chvatalova I, and Jansa J. 2002	<b>Interaction between arbuscular mycorrhizal fungi and cellulose in growth substrate</b> <i>Applied Soil Ecology</i> 19(3): 279–288 [*Vosatka M, The Academy of Sciences of the Czech Republic, Institute of Botany, Pruhonice 25243]

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Smolders A J P,* Echet L, and Roelofs J G M. 2002	<b>The isoetid environment: biogeochemistry and threats</b> <i>Aquatic Botany</i> 73(4): 325–350 [*Smolders A J P, University of Nijmegen, Department of Aquatic Ecology and Environmental Biology, NL-6525 ED Nijmegen, Netherlands]
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Schmidt S* and Stewart G R. 2003	<p><b>delta N-15 values of tropical savanna and monsoon forest species reflect root specialisations and soil nitrogen status</b>  <i>Oecologia</i> <b>134</b>(4): 569–577  [*Schmidt S, University of Queensland, Department of Botany, Brisbane, Queensland 4072, Australia]</p>
Delfin E F, * Paterno E S, Ocampo A M, and Rodríguez F C. 2003	<p><b>Growth, yield and nutrient uptake of mungbean (<i>Vigna radiata</i> L.) grown in antipolo clay amended with lime, nitrogen and phosphorus fertilizers and microbial inoculants</b>  <i>Philippine Agricultural Scientist</i> <b>86</b>(1): 75–83  [*Delfin E F, University of Philippines, Institute of Plant Breeding, College of Agriculture, Physiology Laboratory, Coll 4031, Los Banos, Laguna, Philippines]</p>
Burrows R L* and Pflieger F L. 2002	<p><b>Host responses to AMF from plots differing in plant diversity</b>  <i>Plant and Soil</i> <b>240</b>(1): 169–179  [*Burrows R L, South Dakota State University, Department of Horticulture, Forestry, Landscape and Parks, 201 NPB, Brookings, SD 57007 USA]</p>
Krajinski F, Hause B, Gianinazzi-Pearson V, and Franken P. 2002	<p><b>Mth1, a plasma membrane H<sup>+</sup>-ATPase gene from <i>Medicago truncatula</i>, shows arbuscule-specific induced expression in mycorrhizal tissue</b>  <i>Plant Biology</i> <b>4</b>(6): 754–761  [Franken P, Institute of Vegetable and Ornamental Plants, Theodor Echtermeyer Weg 1, D-14979 Grossbeeren, Germany]</p>
Walter M H,* Hans J, and Strack D. 2002	<p><b>Two distantly related genes encoding 1-deoxy-D-xylulose 5-phosphate synthases: differential regulation in shoots and apocarotenoid-accumulating mycorrhizal roots</b>  <i>Plant Journal</i> <b>31</b>(3): 243–254  [*Walter M H, Leibniz Inst Pflanzenbiochem, Abt Sekundarstoffwechsel, Weinberg 3, D-06120 Halle Saale, Germany]</p>
Karagiannidis N, Bletsos F,* Stavropoulos N. 2002	<p><b>Effect of <i>Verticillium wilt</i> (<i>Verticillium dahliae</i> Kleb.) and mycorrhiza (<i>Glomus mosseae</i>) on root colonization, growth and nutrient uptake in tomato and eggplant seedlings</b>  <i>Scientia Horticulturae</i> <b>94</b>(1–2): 145–156  [*Bletsos F, National Agriculture Research Foundation, Agriculture Research Centre, Macedonia and Thrace, POB 312, Thermi 57001, Greece]</p>
Ronn R,* Gavito M, Larsen J, Jakobsen I, Frederiksen H, and Christensen S. 2002	<p><b>Response of free-living soil protozoa and microorganisms to elevated atmospheric CO<sub>2</sub> and presence of mycorrhiza</b>  <i>Soil Biology &amp; Biochemistry</i> <b>34</b>(7): 923–932  [*Ronn R, University of Copenhagen, Institute of Zoology, Department of Evolutionary Biology, DK-2100 Copenhagen, Denmark]</p>
Steinberg P D and Rillig M C.* 2003	<p><b>Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin</b>  <i>Soil Biology &amp; Biochemistry</i> <b>2003</b> <b>35</b>(1): 191–194  [*Rillig M C, University of Montana, Division of Biology Sciences, Microbial Ecology Program, HS104, 32 Campus Dr 4824, Missoula, MT 59812 USA]</p>
BestelCorre G, DumasGaudot E,* Gianinazzi-Pearson V, and Gianinazzi S. 2002	<p><b>Mycorrhiza-related chitinase and chitosanase activity isoforms in <i>Medicago truncatula</i> Gaertn</b>  <i>Symbiosis</i> <b>32</b>(3): 173–194  [*Dumas-Gaudot E, University of Bourgogne, UMR 1088, INRA, BBCE, IPM, CMSE, BP 86510, F-21065 Dijon, France]</p>

## Forthcoming events

### Conferences, congresses, seminars, symposiums, and workshops

Canada  
10-15 August 2003

#### **The Fourth International Conference on Mycorrhizae (ICOM4)**

ICOM4 Administration  
Bureau des Congrès Universitaires, 6600  
Côte-des-Neiges Road, Suite 215  
Montreal (Quebec) H3S 2A9, Canada

*Fax* (514) 340 4440

*Tel.* (514) 340 3215

*E-mail* icom4@congresbcu.com

Paestum, Italy  
1-6 September 2003

#### **International Wheat Genetics Symposium**

Organizing Secretariat, *Leader s.a.s.*,  
Corso Garibaldi, 148, 84123 Salerno, Italy

*Fax* 0039 089 253238

*Tel.* 0039 089 253170

Melbourne Convention Centre  
28 September - 2 October 2003

#### **ComBio 2003**

Sally Jay Conferences, ComBio2003 Secretariat  
PO Box 2331, Kent Town 5071, South Australia  
(Street address: 2A Athelney Avenue, Hackney 5069 South Australia)

*Fax* (61 8) 8362 0038

*Tel.* (61 8) 8362 0009

Snowbird, Utah  
22-26 October, 2003

#### **Plant Genetics 2003: Mechanisms of Genetic Variation**

American Society of Plant Biologists, 15501 Monona Drive  
Rockville, MD 20855-2768 USA

*Fax* 301 279 2996

*Tel.* 301 251 0560

*E-mail* info@aspb.org

*Web* <http://www.aspb.org/meetings/pg-2003/>

Albacete, Spain  
22-25 October, 2003

#### **1st International Symposium on Saffron Biology and Biotechnology**

Institute for Regional Development  
University of Castilla-La Mancha  
VIAJES S.A., Division Congresos  
Convenciones E Incentivos  
C/ISAAC ALBÉNIZ, 9 (EDIF. MANU II)  
30008 Murcia, Spain

*Fax* +34 968 286417

*Tel.* +34 968 286413 / +34 968 286414