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Research Article

Mycelial Compartments of AM Fungi Spotlights Poly-P and Glomalin Proteins

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ABSTRACT

In this study, the polyphosphate and AM fungal protein contents were computed in the hyphal compartment designed. The compartmentalized pots depicted two sectors namely the root compartment at the centre with a maize plant and the hyphal compartment around the root zone that holded the hyphal components. The experimental set up screened the hyphal components alone that enabled the estimation of various parameters viz., hyphal length, hyphal biomass, hyphal glomalin and polyphosphate contents in the hyphal compartment where, treatments with G. intraradices ranked highest in hypal biomass (28.2 mg cm^{-3}), hyphal glomalin (15.40 µg of BRSP/ mg of hyphae) and hyphal polyphosphate contents (35 and 102 µg of BRSP/ mg of hyphae in extramatical and intraradical hyphae respectively).

Key words: *Compartmentalized pot, Hyphal length, Hyphal glomalin, Extramatical, Intraradical, Polyphosphate.*

INTRODUCTION

Plant growth enhancement due to arbuscular mycorrhizal (AM) fungal colonization has been recognized to result from the supply of absorbed mineral nutrients, particularly phosphorus by extraradical hyphae. It is now widely accepted that phosphate present in the soil is taken up into the extraradical hyphae by a phosphate transporter, subsequently condensed into polyphosphate (poly-P) a key phosphorus compound and translocated by protoplasmic streaming into the intraradical hyphae.

Extraradical mycelium

The extraradical mycelium (ERM) of AM fungi consists of AM fungal hyphae and AM fungal spores that proliferate in the soil with differences in the extent, structure and interconnectedness of mycelial networks[1] holding high correlation with aggregate stability.

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The fungal compartment in soil can be used to study the mycelium[13] and to measure the hyphal phosphate without interference from roots[10]. The methods developed originally by Newman[17] and modified by Tennant[25] initially focused on quantifying root length. Nichols and Wright designed hyphal compartment with nylon mesh bags in the centers of pots with plant roots that provided a root-free hyphal chamber for hyphal extraction.

Intraradical hyphae

Hyphal characteristics of AM fungi acts as a network of stabilizing structures which in addition possess some hyphal proteins called glomalin that glues the soil particles and putsforth a longterm aggregate stability[14]. The extraction of intraradical hyphae from the host roots is possible by enzymatic digestion of root tissue with cellulase and pectinase, followed by hand sorting of the hyphae under a dissecting microscope[8].

Polyphosphates in AM fungal hyphae

Effective P acquisition by the external hyphae is related to three mechanisms namely, a) formation of poly-P in the hyphae thus maintaining low internal phosphate concentrations, b) small hyphal diameter leading to a relatively larger soil volume delivering 'P' per unit surface area compared to the root surface area[12] and a corresponding 2 – 6 times higher P influx rate per unit length of hyphae[11], c) production of extracellular acid phosphatases which catalyse the release of P from organic complexes in the soil. Solaiman *et al*.,[22] used the gridline intersection method (GIM) for measurement of AM fungal hyphae and quantified poly – P in the hyphae extracted from the roots of Onion, colonized by *Gigaspora margarita*.

MATERIAL AND METHODS

A survey was taken up in the sodic soils of Trichy district, located at an altitude of 85 meters above MSL, 10°45' N latitude and 78°36'E longitude at Trichy district, Tamil Nadu and around seven different AM fungal isolates were identified and the purified isolates were used as inoculants (T1-*Acaulospora* sp. T2- *Scutellospora* sp. T3- *Glomus mosseae* T4- *Sclerocystis* sp T5- *Glomus geosporum* T6- *Glomus aggregatum* T7- *Gigaspora* sp) along with an (uninoculated) absolute control for a period of 14 weeks in the compartmentalized pots to study their influence on colonization and hyphal production

Compartmentalized system

A compartmentalized pot experiment was carried out to extract and quantify the extraradical hyphae from the rhizosphere of AM inoculated plants[19]. The sand: soil (2:1 v/v) pot mix was sterilized (121℃ for three consecutive days) and the inoculum was mixed in 1: 10 ratio with the pot mix and transferred to a circular piece of 38-µm nylon mesh (25-30 cm in diameter). This was centered in 1 kg plastic to form the root compartment while sterilized sand was filled in the pots surrounding the mesh to form the hyphal compartment. Maize seeds (COH1MI5 hybrid) were sown in the root compartment and nurtured with Hoagland's nutrient solution. During plant growth, roots were enclosed inside root compartment (nylon mesh), while hyphae penetrated the mesh openings and grew into the hyphal compartment (sand media) (Fig 1).

Extraction of extramatrical hyphae

After growth of about 14 weeks in the nylon bag compartment, the nylon mesh was gently teased from the pots. For extraradical hyphal extraction, the soil from hyphal compartment-I (sand surrounding the nylon bag) were transferred to glass jars with approximately 300 cm^3 per jar. Sterile distilled water was added to each jar to cover sand by approximately 7 cm and vigorously shaken. After several hours the fungal hyphae were found floating on the water surface entangling the soil aggregates (Fig 2). Hyphae were collected by decanting the water over a series of stacked sieves (180, 106 and 45 µ) which were then stained and observed under microscope. Hyphal fraction and fine particles from the 45 µm screen were taken for measurement, quantification, poly-P estimation and glomalin extraction.

Gridline intersect method (GIM)

The hyphae isolated from the soil samples were stored in -20^0 C. Several drops of deionized water were added to the hyphae and the clumps were separated into 1-3 mm length using a scalpel[15]. Hyphae on filters were then dried (60°C, 24 h) and transferred to a Petri plate (5.8 cm diameter). A transparent grid (~66 rectangles each measuring 4 mm x 6 mm) was then placed on top of the Petri plate to form a gridded Petri plate where, the total number of hyphal intersections were counted[9].

$$\textbf{Hyphal length} = \left(\frac{11}{14}\right) \textbf{x grid unit x N}$$

where 'N' in the count of the number of intersections across vertical and horizontal lines and 'grid unit' is the length of the gridded section.

Quantification of extraradical hyphae[2]

The extraradical hyphae present in the root surface and rhizosphere soil were extracted by wet sieving and decantation technique The fresh weight of hyphae was recorded after careful removal of excess moisture with small pieces of filter paper and hyphae were dried at 70^{o}C weighed and expressed in mg g^{-1} of soil.

Isolation of intraradical hyphae[23]

Roots were taken from the root compartment (II) for intraradical hyphal extraction. The extraction involved enzyme digestion and centrifugation of mycorrhizal roots and the supernatant obtained was used for polyphosphate (poly-P) extraction. AM colonized roots were sampled from the pots, thoroughly washed with demineralized water cut into 2 cm bits and stored in sterile 50 ml tube using sterile water. The isolation of intraradical hyphae involves two steps namely a. Disinfection with Pre-treatment and b. Enzyme digestion[5]. The roots were gently pierced with a needle and plates and incubated at 27^0 C for about 15 days. During incubation period, roots were observed for release of fungal structures especially the hyphae. Those fungal structures that were found released into the liquid medium were isolated using sterile forceps and needle under a stereozoom microscope (Leika) and collected for estimation of poly-P.

Organic matter fractions

Particulate Organic Matter (POM) was removed by floatation in a high-density solution. Soil samples (2 g) were covered with a 10 ml of NaCl solution (12 % w/v), vortexed and allowed to settle. After the mineral fraction had settled, the solution was carefully decanted. Floating organic matter (i.e. the POM fraction) was collected on a 0.053 mm sieve. This procedure was repeated four times. The POM fraction collected on the screen was washed with distilled water to remove salt, rinsed from the screen into pre-weighed weigh boats and dried at 70^{o}C whose gravimetric weight was recorded. The settled mineral fraction (i.e. soil minus POM) was washed with distilled water, pelleted by centrifugation, rinsed into pre-weighed weigh boats and dried at 70^{o}C for estimation of humic (HA) and fulvic acid (FA) fractions. After extraction of the POM fraction, HA and FA were co-extracted from the mineral fraction (i.e. soil minus POM) using a multi-step NaOH extraction procedure Swift[24].

Estimation of polyphosphate (poly-P)

The poly-P content was estimated from extraradical as well as from the intraradical hyphae separately as described by Solaiman *et al*.,[22]. The isolated extraradical hyphal samples were first ground using phenol chloroform (PC) mix. The phosphate molecules which comes out from of the hypha into the solution after thorough blending was precipitated by adding Tris-HCl (1 M pH 7.6) and two volumes of acetone. The mix was frozen at -80^0 C for more than 15 min. melted and then centrifuged for 10 min. The residue was air dried and dissolved with water and stored at -20^0 C for performing the assay. The extracts were first treated with RN ase A at 37^0 C for 45 min. followed by addition of 10 µl of poly-P sample to tubes containing 0.75 µl each of acetic acid (0.2 M) and toluidine blue (0.03 %). The amount of poly-P was determined within 15 min. of color development by comparison with a standard curve produced by using 1-5 µg of poly-P glass (Sodiumhexametaphosphate). The poly-P estimation from intraradical hyphae were performed similarly except that

Trichloroacetic acid (TCA) was used as extractant for grinding the hyphae instead of phenol chloroform (PC).

Extraction of hyphal glomalin

Glomalin was extracted from hyphae using 100 m*M* sodium citrate pH 9.0. Extractions were made by autoclaving at 121°C for 1 h similar to extraction method from soil. Extract solution was collected after centrifugation at 10,000 rpm for 3 min. Hyphae was rinsed into weigh boats, dried at 70^0C and weighed. Glomalin concentration (mg g^{-1} hyphae) was measured in each extract solution by Bradford assay and mentioned as Bradford Reactive Soil Protein (BRSP).

RESULTS AND DISCUSSION

Extraradical Hyphal length and biomass

The results of this study showed extraradical hyphae ranging from 0.44±0.03 to 0.98±0.06 m cm^3 soil where *Scutellospora* sp. ranked the highest followed by *Glomus mosseae*. Apart from length, quantification of hyphal structures recorded fresh biomass of about 11.2±0.65 to 20.2±1.17 mg cm^3 soil where *Acaulospora* sp. ranked the highest followed by *Glomus mosseae*. The dried biomass of hyphae recorded dry weight of 4.3 to 6.5 mg g^{-1} where, *Acaulospora* sp. marked the maximum (Table 1). Similar results were given by Miller *et al*.,[16] with external hyphal lengths of 0.80 to 2.70 m cm^{-3} colonized roots and by Hynes *et al*.,[9] who showed 1.19 to 2.93 m cm^{-3} soil. The reports by Singh[21] showed hyphal lengths measured in the soils with mycorrhizal inoculations increased hyphal lengths from 3.2 to 6.2 m cm^{-3} soil and much of the hyphal growth occurred only in the soil that was aggregated. Growth of AM external mycelium was strongly stimulated by organic matter addition to the soil and also the C flow from the plant to the mycelium was higher under organic matter addition. Mineral nutrient addition to the soil also stimulated AM fungal growth[7]. The present study also showed increase in organic matter content and mineral fractions *viz*., humic and fulvic acids (Table 2) in AM fungi inoculated soils which could have contributed to increased hyphal biomass in treatments than the control. Control showed nil fungal structures since the soil was sterilized and kept uninoculated.

Hyphal Glomalin

Glomalin forms a hydrophobic sheath on hyphae that may keep material from being lost from across the hyphal membrane and/or may protect the hyphae from microbial attack[27]. Hyphal traps of *G. intraradices* EY 113/114 sampled from 12-14 weeks from compartmentalized pot culture had glomalin contents ranging from 5-40 µg /cm^2 of the horticultural film[26]. The results of the present study showed BRSP content of glomalin in hyphae of the AM fungus where *Acaulospora* sp. registered the maximum of 11.92 µg of BRSP mg-1 of hyphae. Majority of glomalin (>80%) is tightly bound in fungal hyphae and spores and only autoclaving of the fungal mycelium helps release of glomalin through multiple cycles. It is not simply a cytoplasmic, cell membrane, or mycelial surface-associated protein and this indicates that glomalin is firmly incorporated into the hyphal wall. It is because proteins can be either covalently or non-covalently associate with the fungal cell wall or they can forming either insoluble complexes or being loosely embedded[4,6].

Intraradical hyphae

After an incubation period of about 20 days, the roots under enzyme digestion showed the ooze of mucilaginous secretions (Fig 3) which marked the release of fungal structures. Those root bits which showed mucilaginous ooze out were selected for intraradical hyphal isolation under the microscope. As a result of enzyme digestion, these root bits showed bulging and disruption of root epidermis followed by expulsion and release of hyphae from the root bits (Fig 4) as when analysed under the microscope (10 X) and these intraradical hyphal bits that showed fungal structures (*viz*., vesicles and spores along with the hyphae) were taken for poly-p estimation.

Poly-P contents in the extraradical and intraradical hyphae

The polyphosphate content was analysed in the extraradical mycelium of different AM fungal isolates using Phenol chloroform (PC) (Table 3). In case of extraradical hyphal

extraction, the studies conducted by Solaiman *et al*.,[22] suggested that experiments with Phenol chloroform (PC) method showed leading poly-P contents (102 to 192 µg and 65 to 71 µg respectively) in extraradical mycelium of *Gigaspora margarita* which can be considered supportive to present study showing higher contents (18 to 24 µg) of poly-P with the same extractant. *Scutellospora* sp. recorded the maximum poly-P content of 24±1.52 µg followed by *Acaulospora* sp. with 23±1.46 µg g-1 of hyphae.

The poly-P content of the intraradical mycelium was extracted using Trichloroacetic acid (TCA). The poly-P content recorded a highest of 85±0.98 µg in *Scutellospora* sp. that was on par with T4. This matches with the data of Solaiman *et al*.,[22] who showed higher polyphosphates of 59 to 137µg under TCA method. The poly-P content of the intraradical mycelium in total was approximately five folds higher when compared to that of extraradical mycelium.

Correlation study

The hyphal biomass yielded through compartmentalized system was in more positive correlation with that of hyphal glomalin content (r=0.74) (Fig 5) and the supportive literatures are cited here. Glomalin in soil being recalcitrant in nature helps to stabilize aggregates which in turn help to physically protect organic matter within aggregates from degradation and appears to be highly correlated with stability of the aggregates[27,3]. This is demonstrated in a number of experiments where, soil aggregation was positively correlated with Total Extractable Glomalin (0.89) and Easily Extractable Glomalin (0.90) in both agricultural and native soils[20]. Analogous to these findings, the correlation analysis worked out between the hyphal biomass and hyphal glomalin contents in this study illustrated that a strong positive correlation existed between these two components and still confirmed that the BRSP proteins detected were surely the glomalin related soil proteins of AM fungi.

Table 1: Quantification of extramatrical hyphae extracted through compartmentalized pot experiment

Samples	Length of hyphae (m cm^{-3} soil)	Hyphal biomass (mg cm^{-3} soil)	Dry weight of AM hyphae (µg cm^{-3} soil)	Hyphal Glomalin (µg of BRSP mg^{-1} of hyphae)
T1	0.70±0.04	20.2±1.17	6.5±0.38	11.92±0.69
T2	0.98±0.06	18.3±1.06	6.1±0.36	11.73±0.68
T3	0.85±0.05	19.3±1.11	6.2±0.36	11.88±0.69
T4	0.71±0.04	15.5±0.89	5.0±0.29	10.36±0.60
T5	0.57±0.03	15.7±0.91	5.7±0.33	10.68±0.62
T6	0.44±0.03	11.2±0.65	4.9±0.29	10.55±0.61
T7	0.49±0.03	13.8±0.80	4.3±0.25	10.65±0.61
Control	-	-	-	-
S.Ed	0.078	1.638	0.554	1.083
CD (0.05)	0.165	3.417	1.165	2.275

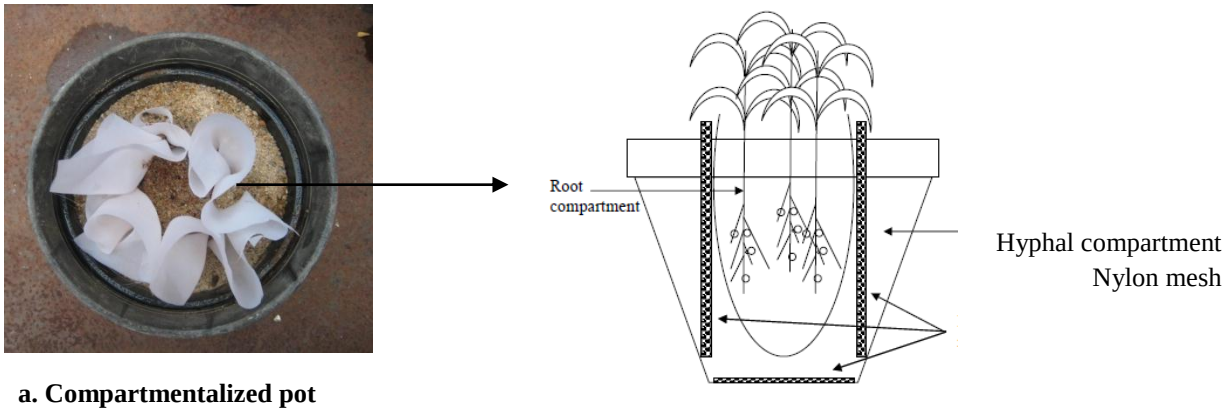
Table 2: Particulate Organic matter (POM) and mineral fractions in compartmentalized pot experiment soil

Treatments	Particulate organic matter (mg g^{-1})	Mineral fraction	
		Humic acid (mg g^{-1})	Fulvic acid(mg g^{-1})
T_1	47.5±1.37	35±2.02	28±1.61
T_2	37.5±1.08	20±1.15	25±1.44
T_3	35.0±1.01	40±2.33	24±1.38
T_4	25.0±0.72	25±1.44	18±1.04
T_5	37.0±1.08	30±1.73	18±1.05
T_6	27.5±0.79	15±0.88	18±1.06
T_7	32.5±0.93	25±1.44	20±1.15
T_8	20.0±0.57	12±1.70	12±0.70
S.Ed	3.44	2.76	2.19
CD (0.05)	7.18	5.76	4.57

Table 3: Assessment of polyphosphate (poly – P) content in hyphae of AM fungal isolates extracted through compartmentalized pot experiment

Samples	Polyphosphate content of AM hyphae (µg g^{-1} of hyphae)	
	Extraradical mycelium (ERM)	Intraradical mycelium (IRM)
T1	23±1.46	70±0.81
T2	24±1.52	85±0.98
T3	22±1.40	75±0.87
T4	18±1.14	85±0.98
T5	19±1.21	78±0.90
T6	19±1.21	84±0.97
T7	20±1.27	59±0.68
Control	-	-
S.Ed	2.150	7.454
CD (0.05)	4.518	15.661

T1- *Acaulospora* sp; T2- *Scutellospora* sp; T3- *Glomus mosseae*; T4- *Sclerocystis* sp; T5 - *Glomus geosporum*; T6- *Glomus aggregatum*; T7- *Gigaspora* sp. Control – Uninoculated soil

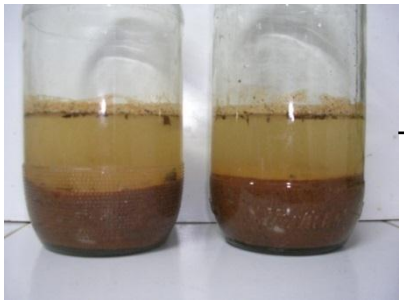


a. Compartmentalized pot

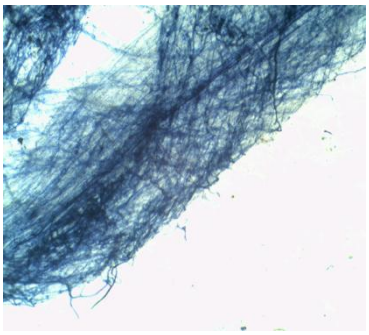
b. Compartmentalized pots with maize grown under controlled condition

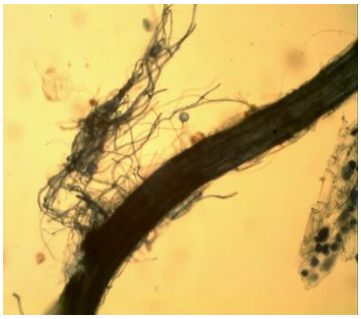
Fig. 1: Compartmentalized pot experiment for extraction of AM fungal hyphae

a. Soil from hyphal compartment



Soil aggregates with hyphae floating on surface

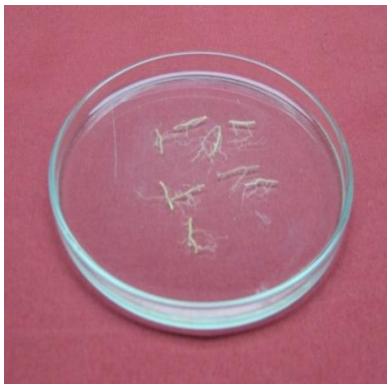




b. Interwoven hyphae (40 X) **c. Extramatical hyphae (10 X)**

Fig. 2: Extraction of extramatical hyphae of AM fungi from compartmentalized pot experiment

a. Mycorrhizal roots under enzymatic digestion **b. Root bits showing release of exudates**

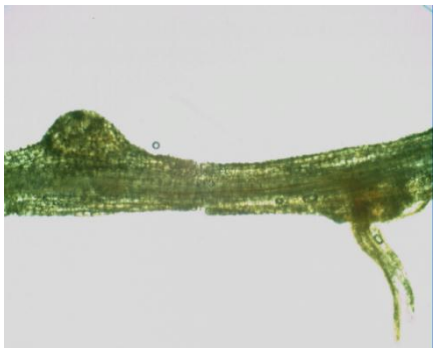


c. Isolation of digested root bits

Fig. 3: Enzymatic digestion for extraction of intraradical hyphae

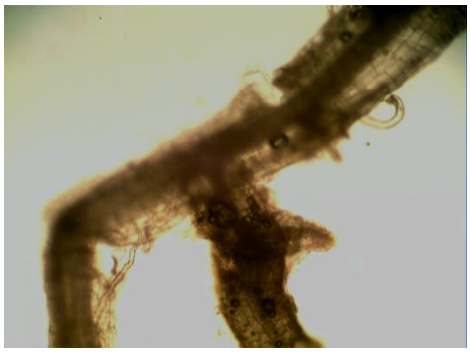
a. Disruption of root epidermis after enzyme digestion

b. Expulsion of fungal structures



hyphae

c. Release of hyphae from a root bit during digestion



spores

Fig. 4: Release of intraradical hyphal structures of AM fungi at 15 days after Enzyme digestion (10X)

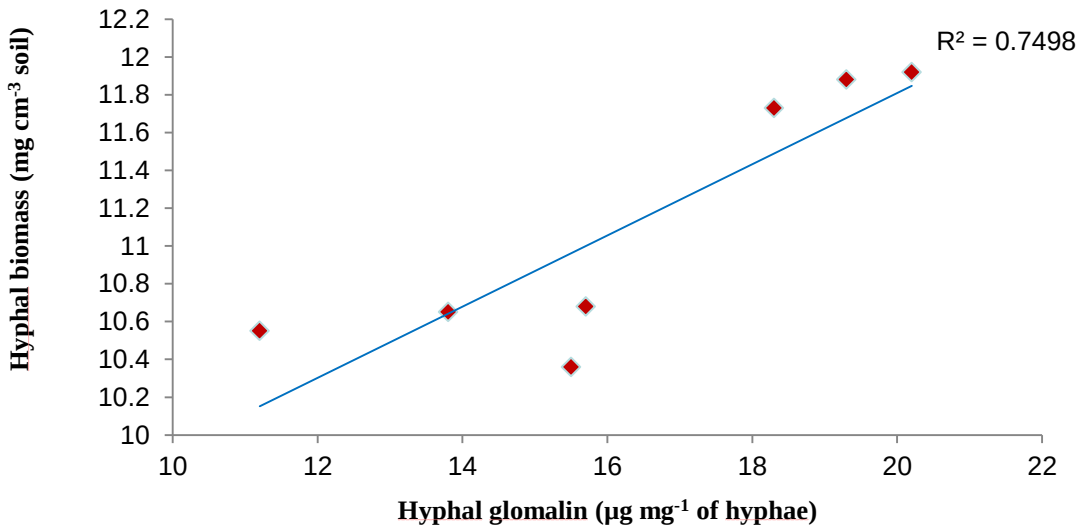


Fig. 5: Correlation analysis of hyphal biomass and hyphal glomalin contents

CONCLUSION

Results of the present study marked that, *Acaulospora* sp performed the best in case of producing maximum hyphal biomass and glomalin content. In case of polyphosphate production *Scutellospora* sp. was dominating in extraradical as well as intraradical hyphal production. Other than *Acaulospora* sp. and *Scutellospora* sp. treatments, it was *Glomus mosseae* which was almost on par and the second best sometimes. Preliminary studies on the diversity registered predominance of these above mentioned isolates in their native rhizosphere and this underlined their strong survival ability either in terms of spores or germinated hyphal structures and thus concluded that these would be potential isolates with intensive hyphal biomass, hyphal protein and poly-P production not only under gnotobiotic conditions but also under *in vivo* conditions. The ability of the AM fungal isolates to enhance Glomalin concentration and to form extensive and dense mycelial networks thereby influencing soil aggregate stability suggests the possibility of selecting the most efficient isolates to be utilized for soil quality improvement and land restoration programs.

REFERENCES

1. Avio, L., Pellegrino, E., Bonari, E. and Giovannetti, M., Functional diversity of arbuscular mycorrhizal fungal isolates in relation to extraradical mycelial networks. *New Phytologist* **172:** 347-357 (2006).
2. Bethlenfalvay, G. J. and Ames, R. N., Comparison of two methods of quantifying extraradical mycelium of vesicular-arbuscular mycorrhizal fungi. *Soil Science Society* of America *Journal* **51:** 834–837 (1987).
3. Bird, S. B., Herrick, J. E., Wander, M. M. and Wright, S. F., Spatial heterogeneity of aggregate stability and soil carbon in semi-arid rangeland. *Environmental Pollution* **116:** 445-455 (2002).
4. Carlile, M. J., Watkinson, S. C. and Gooday, G. W., *The Fungi*, Second ed. *Academic Press*, London. pp. 578 (2001).
5. Cranenbrouck, S., Voets, L., Celine, B., Renard, L., Strullu, D. G. and Declerck, S., Methodologies for *in vitro* cultivation of Arbuscular Mycorrhizal fungi with root organs. **4:** In: *In vitro culture of mycorrhizas* (Eds.) Declerck, S., Strullu, D. G., Fortin, A., pp. 363-365 (2005).
6. de Vries, O. M. H., Fekkes, M. P., Wosten, H. A. B. and Wessels, J. G. H., Insoluble hydrophobin complexes in the walls of Schizophyllum commune and other filamentous fungi. *Archives of Microbiology* **159:** 330-335 (1993).
7. Hammer, E., *Nutrient balance and salinity stress in arbuscular mycorrhizal fungi*. Doctoral thesis Lund University Science. Section of Biological and Earth Sciences. *Department of Ecology*. Microbial Ecology. Lund, Sweden (2010).
8. Hepper, C. M., Sen and Maskall, C. S., Identification of vesicular arbuscular mycorrhizal fungi in roots of leek (*Allium porrum* L.) and maize (*Zea mays* L.) on the basis of enzyme mobility during polyacrylamide gel electrophoresis. *New Phytologist* **102:** 529-539 (1986).
9. Hynes, M. M., Zasoski, R. J. and Bledsoe, C. S., Evaluation of Two Techniques for Quantification of Hyphal Biomass. In: *Proceedings of the sixth California oak symposium: Today's challenges, tomorrow's opportunities***.** General Technical Report. (Eds.) Merenlender, Adina, Mc Creary, Douglas, Purcell, Kathryn, L. and Albany, C. A., *US Department of Agriculture*, Forest Service, *Pacific Southwest Research Station*: pp. 139-148 (2006).
10. Jakobsen, I., In: *Transport of phosphorus and carbon in VA mycorrhizas* (Eds.), Varma, A. and Hock, B., Mycorrhiza Springer, Berlin, pp. 297- 324 (1995).
11. Jakobsen, I., Abbott, K. and Robson, A. D., External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. I. Spread of hyphae and phosphorus inflow into roots. *New Phytologist* **120:** 371-380 (1992).

12. Jungk, A. and Claassen, N., Availability in Soil and Acquisition by Plants as the Basis for Phosphorus and Potassium supply to Plants. *Journal of Plant Nutrition and Soil Science* **152(2):** 151-157 (1989).
13. Kothari, S. K., Marschner, H. and Ramheld, V., Contribution of VA mycorrhizal hyphae acquisition of P and Zn by maize growth in calcareous soil. *Plant and Soil* **131:** 177-185 (1991).
14. Miller, R. M. and Jastrow, J. D., Mycorrhizal fungi influence soil structure. In: *Arbuscular Mycorrhizas: Molecular Biology and Physiology*. (Eds.) Kapulnik Y and Douds D D. *Kluwer Academic*, Dordrecht, pp. 3-18 (2000).
15. Miller, R. M. and Jastrow, J. D., *Extraction and quantification of external mycorrhizal hyphae* (for soils dominated by silts and clays or high in particulate organic matter). *Argonne National Laboratory* (2006).
16. Miller, R. M., Reinhardt. D. R. and Jastrow, J. D., External hyphal production of vesicular-arbuscular mycorrhizal fungi in pasture and tallgrass prairie communities. *Oecologia* **103:** 17-23 (1995).
17. Newman, E. I. A., method of estimating the total length of root in a sample. *J. Appl. Ecol.,* **3:** 139-145 (1966).
18. Nichols, K. A. and Wright, S. F., Contributions of soil fungi to organic matter in agricultural soils. In: *Functions and Management of Soil Organic Matter in Agroecosystems***.** (Eds.) *MagdoffWeil F R, CRC Press, Boca Raton*, FL, pp 179-198 (2004).
19. Nichols, K. A., *Characterization of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi*. Ph.D. Thesis, *University of Maryland, College of Agriculture and Natural Resources*, College Park, MD (2003).
20. Rillig, M. C., Ramsey, P. W., Morris, S. and Paul, E. A., Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. *Plant and Soil* **253:** 293-299 (2003).
21. Singh, S., Role of mycorrhiza in disturbed lands. Part ll. Soil compaction, soil erosion, soil aggregation, and volcanic eruptions *Mycorrhiza News* **15(2):** 2-11 (2003).
22. Solaiman, M. Z., Ezawa, T., Kojima, T. and Saito, M., Polyphosphate in intraradical and extraradical hyphae of an Arbuscular Mycorrhizal fungus, *Gigaspora margarita*. *Appl. Environ Microbiol*, **65(12):** 5604-5606 (1999).
23. Solaiman, M. Z. and Saito, M., Use of sugars by intraradical hyphae of arbuscular mycorrhizal fungi revealed by radiorespirometry. *New Phytologist* **136:** 533–538 (1997).
24. Swift, R. S., Organic matter characterization. Methods of Soil Analysis. In: Part 3. Chemical methods. (Eds.), D. L. Sparks et al. SSSA Book Series, no. 5 Madison, WI. pp. 1018-1020 (1996).
25. Tennant, D. A., test of a modified line intersect method of estimating root length. *The Journal of Ecology* **63:** 995-1001 (1975).
26. Wright, S. F. and Upadhyaya, A., Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. *Mycorrhiza* **8:** 283-285 (1999).
27. Wright, S. F. and Upadhyaya, A., A survey of soils for aggregate stability and glomalin, a glycoproteins produced by hyphae of arbuscular mycorrhizal fungi. *Plant and Soil* **198:** 97-107 (1998).