Mycorrhizal Biodiversity in Some Grass Species of Anamalai Hills, Tamilnadu, India

Rehana banu, H[b], N. Nagarajan[a]
[a]PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641 029, Tamilnadu, India
[b]Department of Botany, Nirmala College for Women, Coimbatore -641018, Tamilnadu, India
Corresponding author:
Rehana banu, H
Department of Botany, Nirmala College for Women, Coimbatore -641018, Tamilnadu, India

Abstract

Arbuscular mycorrhizal colonization in the roots and spore numbers in the rhizosphere of thirty five different grasses from Anamalai Hills, Tamilnadu, India were investigated. Percentage of fungal infection in the roots was analyzed by a staining method of Phillip and Hayman. Quantity of mycorrhizal spores was determined by employing wet-sieving and decanting technique.The results indicated that all the grasses examined during the study, exhibited the presence of arbuscular mycorrhizal association. The percentage of root colonization by AM fungi varied from 14% to 68%. *Perotis indica* had maximum percentage of colonization (68%). The presence of greater number of spore in soil was always associated with the incidence of abundant mycelia. Number of mycorrhizal fungus spores ranged between 172 to 475 per 100g air dried soil. A total of 26 arbuscular mycorrhizal fungal spores were isolated from the grasses represented by four genera, namely 6 species of *Acaulospora*, 4 species of *Gigaspora*, 14 species of *Glomus* and 2 species of *Scutellospora.* The frequency of mycorrhizal fungus infection showed positive correlation with soil pH, moisture, water holding capacity, texture, total nitrogen, phosphorus, calcium, potassium, and magnesium. Especially phosphorus and nitrogen in the soil greatly influenced the plant root infection by AM fungi.

KEYWORDS: Mycorrhizal colonization, rhizosphere, percentage of root colonization and positive correlation.

INTRODUCTION

German Botanist Frank (1885) coined the term mycorrhizae for the first time to designate the symbiotic relationship between the fungi and plant roots. Since then scientists started exploiting them for the welfare of mankind. Mycorrhizas are ecologically important symbioses in which the fungi derive photosynthetic sugars from their plant hosts, which in turn benefit from the fungus-mediated uptake of mineral nutrients (Marc Ducousso *et al*., 2008). Depending on the relative arrangements of the fungus and the root, mycorrhizal symbioses have been classified into two major types: endomycorrhizas and ectomycorrhizas (ECM). Within endomycorrhizas, arbuscular mycorrhizas (AM) are presumed to have been crucial in the colonization of the land by plants (Heckman *et al*., 2001). AM fungi are obligate symbionts with plant roots, which help in nutrient mobilization and consequently, plant grown. There are two phases of AM fungi – one is intraradical (inside roots) and the other is extraradical (outside the roots). The intraradical phase involves the

arbuscules (site of nutrient exchange), vesicles (storage organ), and mycelium. The extraradical phase contains extraradical hyphae and spores (Gupta *et al*., 2009).

AM fungi belonging to the Phylum Glomeromycota are geographically ubiquitous occur over a broad ecological range including associated agriculture, horticulture, pasture grasses, tropical plants and cereals (Qadri, 2004; Peterson *et al*., 2004). AM symbiosis that appeared with the first land plants more than 400 million years ago, is still formed by the large majority of extent plant species with no host specificity (Redecker *et al*., 2000). AMF increase the effective absorptive area of roots by formation of an extensive extraradical hypha network that enhances efficiency in absorption of nutrients (George, 2000). These fungi are known to increase the nutritional status of the host, particularly that of phosphorous, and thereby enhance their growth, development and yield (Bagyaraj and Verma, 1995; Bagyaraj, 2007).

The reports of mycorrhiza associated with grasses of Anamalai Hills are not available and studied. The main objectives of the present investigation was an attempt to study the occurrence of AM fungi in the grasses of Anamalai Hills, Tamilnadu, India by determining the extent of root colonization, spore density in the rhizospheric soil and the actual species composition associated with each host.

MATERIALS AND METHODS

Soil and plant root sampling:

Roots from 35 different grasses belonging to the family Poaceae collected from Anamalai Hills, Tamilnadu, India were examined for mycorrhizal infection. Five rhizosphere soil samples dug up to a depth of 10 cm, were collected from each plant species after removing the surface soil and litter covering. These samples were kept in sterilized bags and were transported to the laboratory immediately after collection and were stored in deep freezer at 4ºC until use.

a) Processing of roots for assessing the existence of root colonization:

The roots were cleared and stained in tryphan blue with a modified version of the Philips and Hayman's (1970) method. Roots were cut into 1-2 cm pieces, heated at 90ºC in 10% KOH for about 1 hour. The stained roots were examined under compound microscope (X40 – X100) for mycorrhizal infection. A root segment was considered to be positive if it showed mycelium, vesicle or arbuscle or any other combination among them. The extent of AM colonization was estimated by the percentage of root length colonized by mycorrhiza (Giovannetti and Mosse 1980).
The percentage of root colonization were calculated using the following formula:

$$\% \text{ root colonization} = \frac{\text{Number of positive segments}}{\text{Total number of segments studied}} \times 100$$

b) Extraction of spores:

Spores were recovered from soil samples by the wet-sieving and decanting method (Gerdemann and Nicolson, 1963). From each soil sample, 100g soil was taken and mixed with 1: 1 of luke warm water in a large beaker until all the aggregates dispersed to leave a uniform suspension. To remove organic matter and roots, the suspension was decanted through a 710µm sieve. The suspension that passed through 710µm was decanted through 425 µm, 250 µm, 150 µm, 75µm and 45µm sieves

consecutively to collect spores of different sizes. The AM fungal spores collected on filter paper (Whatman filter paper No.1) after wet sieving and decanting they were observed under stereoscopic binocular microscope. These spores were picked through needle and mounted on glass slide in lactophenol mountant (Koske and Tessier, 1983).

c) Identification of AM fungi:

AM spore identification was done by using the "Manual for the identification of VA mycorrhizal fungi" of Schenck and Perez (1990). Classification was based on colour, size, shape, surface, structure, general nature of the spore contents and hyphal attachment. To know the relationship between the soil properties with AM colonization different physical and chemical properties of soil were analyzed. Soil sample were analyzed for pH, total nitrogen, phosphorus, calcium, potassium and magnesium following the standard procedures as described (Piper, 1966).

RESULTS

In the present study, the survey of grasses for AM fungi showed variability in colonization and spore density. All the grasses selected for study exhibited the presence of AM fungal association. Hyphal stages of colonization were seen in all the grasses. The colonization was observed in the form of mycelium, arbuscules, vesicles and chlamydospores. However, vesicular and arbuscular colonization were seen only in few grasses. The maximum spore population was displayed in the plant species of *Cynodon dactylon* (475/100g of soil) and minimum spore population was observed in *Chrysopogon asper* (172/100g of soil).

The highest AM fungal infection found in the roots of *Perotis indica* (68%) while the lowest fungal infection was noticed in *Bothriochloa pertusa* (14%). During the course of study, totally 26 arbuscular mycorrhizal fungal species belonging to 4 genera namely *Acaulospora* with 6 species, *Gigaspora* with 4 species, *Glomus* with 14 species and *Scutellospora* with 2 species were isolated (Table 1).

AM fungi are common symbiotic associates of most tall grass prairie plants (Gibson and Hetrick, 1988). A high diversity of AM fungal species is found in tall grass prairie (Hetrick and Bloom, 1983; Anderson *et al*., 1984; Liberta and Anderson, 1986). The rhizosperic soil collected from all the locations was more or less neutral to alkaline. In this soil, *Glomus* spp is the most dominant fungi and frequently observed. *Glomus* is associated with all the host species. The dominance of *Glomus* species in alkaline soil was also reported by many workers (Mosse, 1973; Gautam *et al*., 2009).

Cynodon dactylon roots were fairly colonized with mycorrhiza showing 64, 6 and 33% mycelial, arbuscular and vesicular colonization, respectively in non-rhizospheric soil of *Narcissus papyraceus* (Javaid *et al*., 2007). AM fungal infection in the grasses of *Bothriochloa pertusa* (70%), *Brachiaria reptans* (0%), *Chloris dolichostachya* (25%), *Cynodon dactylon* (50%), *Dactyloctenium aegyptium* (60%), *Eleusine indica* (75%), *Eragrostis ciliaris* (100%), *Setaria glauca* (20%) and *Setaria tomentosa* (0%) in and around Delhi University (Rajni Gupta and Mukerji, 1996). In *Brachiaria reptans* and *Setaria tomentosa* AM colonization was seemingly absent; in most other grasses arbuscles and vesicles were present. In the present findings, *Bothriochloa pertusa* (14%), *Cynodon dactylon* (53%), *Dactyloctenium aegyptium* (25%), *Eleusine indica* (35%) observed the varied AM fungal infection.

The other species of *Brachiaria distachya* (58%), *Chloris barbata* (47%), *Eragrostis tenella* (60%), *Setaria intermedia* (32%) and *Setaria verticillata* (22%) showed the AM infection. The variation between the two findings may be due to climatic factors. The population of AMF in soil is also dependent on the factors like climate, soil and the host plant community (Hayman, 1982; Brundett, 1991; Dehne, 1987). Renker *et al*. (2005) analysed the AM fungal infection in the plant species of *Poa pratensis* and they concluded that the rate of infection not determined. But they reported the other species *Arrhenathermum elatilus* 33% and *Elynus repens* (24.0±10.5) belongs to the family Poaceae. But in the present study observed that the another species *Poa annua* showed infection and spore population is 42 and 315/100gm of soil respectively.

Sadiq Gorsi (2002) reported the arbuscular mycorrhizal infection in *Cymbopogon citratus* (50%) and *Triticum aestivum* (60%) belongs to the family Poaceae at the area of Azad Kashmir. In the present study reveals that the *Cymbopogon citratus* 29% colonization was observed and the cleaned roots showed hyphae and intracellular arbuscles and vesicles. Sampath kumar *et al*. (2006) observed the AM fungal infection (30%) in Poaceae member of *Cynodon dactylon* and the spore population (1200/100gm of soil) from Thanjavur district. The similar finding was recorded in the present study of Poaceae member *Cynodon dactylon*. The AM fungal infection and spore population was 53% and 475/100gm of soil respectively.

The results of this investigation clearly indicate that most of the plants belonging to the family Poaceae showed AM fungal infection. The degree of AM formation varied in all the plants.

Conclusion:

The result obtained from the study suggests that the colonization percentage and number of AM fungal spores differ with different grass species. Among the four genera, *Glomus* spp was found much more frequent than other genera. Therefore based on this preliminary investigation, it was not possible to assess the host specificity in detail of other medicinal plants to AM fungi colonization. It is therefore concluded that there is a bright scope for further detailed study for understanding the host specificity of AM fungi species on the other plant species and their effect on enhancement of secondary metabolites active principles.

TABLE 1: AM fungal infection, spore population and AM fungi in the rhizospheric soil of grass species collected in and around the areas of Anaimalai hills.

S. No	Name of the plant species	% of infection	Spore population per 100 gm soil	VAM fungal spores
1.	*Andropogon pumilus* Roxb.	31	200	*Acaulospora sporocarpa* *Gigaspora rosea* *Glomus fasciculatum*
2.	*Apluda mutica*, L.	32	240	*Glomus boreale* *Gl.canadense* *Gl.deserticola* *Gl.multicaule*

3.	*Aristida adscensionis*, L.	26	270	*Gigaspora decipiens* *Glomus etunicatum* *Gl.hoi*
4.	*Bothriochloa pertusa* (L.) A.Camus	14	190	*Glomus citricola* *Gl.flavisporum* *Gl.pulvinatum*
5.	*Brachiaria ramosa (L.) Stapf in Oliver*	32	300	*Acaulospora undulata* *Glomus etunicatum*
6.	*Brachiaria distachya* (L.) Stapf in Oliver	58	321	*Gl.flavisporum* *Gl.hoi* *Glomus multicaule*
7.	*Cenchrus ciliaris*, L.	28	180	*Gl.etunicatum* *Glomus fasciculatum* *Scutellospora auriglobosum*
8.	*Chloris barbata*, Sw.	47	276	*Glomus boreale* *Gl.delhiense* *Gl.geosporum*
9.	*Chrysopogon aciculatus* (Retz.) Trin.	34	200	*Acaulospora rehmii* *Gl.deserticola* *Gl.pulvinatum*
10.	*Chrysopogon asper* Heyne ex Hook. f.) Blast. & McCann.	26	172	*Gigaspora decipiens* *Glomus canadense* *Gl.geosporum*
11.	*Chrysopogon fulvus* (Sprengel) Chiov.	39	250	*Gigaspora albida* *Glomus fasciculatum* *Gl.hoi*
12.	*Cymbopogon citratus* (DC) Stapf.	29	310	*Acaulospora rehmii* *Gigaspora albida* *Glomus etunicatum* *Gl.invermayanum*
13.	*Cynodon dactylon* (L.) Pers.	53	475	*Acaulospora thomii* *Gigaspora margarita* *Glomus citricola* *Gl.fasciculatum* *Gl.flavisporum*
14.	*Dactyloctenium aegyptium* (L.) P.Beauv.	25	285	*Acaulospora denticulata* *Glomus geosporum* *Gl.pulvinatum*
15.	*Digitaria bicornis* (Lam.) Roemer ex Schultes.	42	329	*Glomus fasciculatum* *Gl.boreale* *Gl.multicaule*
16.	*Digitaria longiflora* (Retz.) Pers.	27	298	*Glomus canadense* *Gl.delhiense* *Gl.deserticola*
17.	*Digitaria ternata* (A.Rich.) Stapf ex Pyer in Harv & Sonder.	39	360	*Glomus ambisporum* *Gl.fasciculatum* *Scutellospora nigra*

18.	*Echinochloa colona* (L.) Link.	23	239	*Acaulospora undulata* *Gigaspora rosea* *Glomus delhiense*
19.	*Eleusine indica* (Linn.) Gaertn.	35	190	*Gigaspora albida* *Gig.decipiens* *Glomus fasciculatum* *Gl.hoi*
20.	*Enteropogon monostachyos* (Vahl) Schumann ex Engl.	27	240	*Glomus boreale* *Gl.fasciculatum* *Gl.flavisporum*
21.	*Enneapogon elegans* (Steudel) Stapf.	32	280	*Glomus canadense* *gl.deserticola* *Scutellospora nigra*
22.	*Eragrostiella bifaria* (Vahl) Bor.	38	240	*Acaulospora rehmii* *Glomus invermayanum* *Gl.multicaule*
23.	*Eragrostis nigra* Nees ex Steudel.	39	322	*Acaulospora denticulata* *Glomus etunicatum* *Gl.fasciculatum*
24.	*Eragrostis tenella* (L.) P.Beauv ex Roemer & Schultes.	60	336	*Gigaspora albida* *Gig.decipiens* *Glomus pulvinatum*
25.	*Heteropogon contortus* (L.) Beauv ex Roem & Schult.	46	260	*Acaulospora rehmii* *Glomus boreale* *Gl.delhiense* *Gl.invermayanum*
26.	*Panicum psilopodium* Trin.	56	320	*Glomus fasciculatum* *Gl.geosporum* *Scutellospora auriglobosum*
27.	*Panicum repens* Linn.	25	210	*Gigaspora rosea* *Glomus fasciculatum* *Gl.flavisporum* *Gl.hoi*
28.	*Paspalidium flavidum* (Retz.) A.Camus in Lecomte.	22	200	*Acaulospora scrobiculata* *Glomus pulvinatum* *Scutellospora nigra*
29.	*Perotis indica* (L.) Kuntze.	68	475	*Glomus ambisporum* *Gl.citricola* *Scutellospora nigra*
30.	*Poa annua* L.	42	315	*Acaulospora rehmii* *Gigaspora decipiens* *Glomus ambisporum*
31.	*Sacciolepis indica* (L.) Chase.	37	293	*Acaulospora undulata* *Glomus citricola* *Gl.etunicatum*
32.	*Setaria intermedia* (Roth) Roemer & Schultes.	32	176	*Acaulospora sporocarpia* *Glomus boreale* *Gl.geosporum*

33.	*Setaria verticillata* (L.) P.Beauv	22	216	*Gigaspora albida* *Glomus fasciculatum*
34.	*Sporobolus wallichii* Munro ex Trin.	35	183	*Glomus canadense* *Gl.fasciculatum* *Scutellospora auriglobosum*
35.	*Urochloa panicoides* P.Beauv.	50	295	*Acaulospora scrobiculata* *Glomus delhiense* *Gl.invermayanum*

Table 2: Physicochemical properties of rhizosphere soil samples of grass species at Anamalai Hills.

S. No	Name of the plant	pH	Macronutrients Kg/ha			Micronutrients (ppm)			
			N	P	K	Iron	Zinc	Mn	Cu
1.	*Andropogon pumilus*	6.2	513	12.9	542	16.3	2.6	11.6	0.19
2.	*Apluda mutica*	4.8	523.5	12.1	568	19.4	2.1	10.5	0.27
3.	*Aristida adscensionis*	4.0	420	12.4	538	20.5	3.25	12.4	0.23
4.	*Bothriochloa pertusa*	5.8	524.3	15.3	526	21.3	1.23	11.1	0.18
5.	*Brachiaria ramosa*	4.6	532.1	13.2	511	22.6	2.5	10.7	0.13
6.	*Brachiaria distachya*	4.9	427.5	12.6	542	21.6	1.45	13.85	0.33
7.	*Cenchrus ciliaris*	4.4	511	12.5	526	20.3	2.4	11.5	0.28
8.	*Chloris barbata*	7.5	562	14.8	505	19.6	3.56	11.3	0.36
9.	*Chrysopogon aciculatus*	5.5	488	12.7	528	17.6	1.9	12.3	0.16
10.	*Chrysopogon asper*	5.9	516.2	11.1	539	21.5	1.7	14.9	0.22
11.	*Chrysopogon fulvus*	6.4	562.7	13.2	553	20.8	2.8	8.5	0.29
12.	*Cymbopogon citratus*	5.3	552	11.9	534	16.5	2.0	10.8	0.14
13.	*Cynodon dactylon*	6.2	519	11.6	543	22.1	1.40	11.4	0.22
14.	*Dactyloctenium aegyptium*	4.5	484	12.6	497	20.6	3.52	12.2	0.25
15.	*Digitatia bicornis*	5.2	542	12.2	522	19.2	1.05	12.98	0.20
16.	*Digitaria longiflora*	4.7	493	14.2	519	19.7	2.72	10.9	0.31
17.	*Digitaria ternata*	5.0	565.2	12.3	510	13.5	3.3	12.5	0.15
18.	*Echinochloa colona*	7.2	510	11.8	543	21	2.9	13.2	0.35
19.	*Eleusine indica*	6.5	562.2	12.8	516	14.9	2.62	13.64	0.11
20.	*Enneapogon elegans*	5.6	535.5	10.5	532	19.3	2.03	8.9	0.35
21.	*Enteropogon monostachyos*	6.0	552.1	11.3	520	17.2	3.37	9.78	0.21
22.	*Eragrostiella bifaria*	6.1	570.1	12.9	545	22.0	1.65	14.3	0.24
23.	*Eragrostis nigra*	5.3	506	11.7	558	16.9	2.42	10.0	0.30
24.	*Eragrostis tenella*	7.4	554.4	12.2	541	20.2	1.26	11.2	0.38
25.	*Heteropogon contortus*	7.9	539.2	9.2	520	13.9	1.56	12.37	0.18
26.	*Panicum psilopodium*	5.7	480.5	14.5	515	22.5	2.2	6.8	0.10
27.	*Panicum repens*	6.4	546.3	9.5	540	14.4	3.20	13.58	0.17
28.	*Paspalidium flavidum*	4.8	490	11.0	517	17.1	1.09	7.72	0.26
29.	*Perotis indica*	7.2	504	10.6	533	19.9	2.23	8.4	0.17
30.	*Poa annua*	6.3	512	9.7	554	18.7	2.69	12.20	0.32
31.	*Sacciolepis indica*	7.3	551.3	12.0	514	23	1.29	9.2	0.39
32.	*Setaria intermedia*	6.9	513	14.9	562	14.3	3.38	14.57	0.19

33.	*Setaria verticillata*	7.4	551.2	11.2	523	20.7	2.5	10.98	0.37
34.	*Sporobolus wallichii*	4.7	542.3	9.8	567	19.0	2.12	8.43	0.34
35.	*Urochloa panicoides*	5.5	540	14.4	504	17.4	1.26	13.76	0.32

REFERENCES:

Anderson, R.C., Liberta A.E., and Dickman L.A. (1984). Interaction of vascular plants and vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. Oecologia. 64: 111-117.

Bagyaraj, D.J. (2007). Arbuscular Mycorrhizal fungi and their role in horticulture. In: Recent trends in horticultural biotechnology. Eds., Keshavchandran, R *et al*., pp: 53-58.

Bagyaraj, D.J., and Varma A. (1995). Interactions between Arbuscular Mycorrhizal fungi and plants: their importance in sustainable agriculture in arid and semiarid tropics. Advances in Microbial Ecology. 14: 119-142.

Brundett, M. (1991). Mycorrhizas in natural ecosystems. Advances in Ecological Research. 21: 171-313.

Dehne, H.W. (1987). Management of VA mycorrhizae in temperate crops. In: Mycorrhizae in the Next Decade- Practical Appliations and Research priorities, Proc. 7th NACOM, Greenvillae, Florida, 3-8 May, USA.

Frank, A.B. (1885). Neue Mitteilungen ube" die mycorrhiza der Baume und der Monotropa hypopitys. Ber. Deut. Bot. Ges. 3: 27-33.

Gautam, N.K. and Roy A.K. (2009). Seasonal Diversity in Arbuscular Mycorrhizal Fungi associated with some medicinal plants of Jharkhand, J. Indian Bot. Soc., 88(1&2): 56-59.

George, E. (2000). Nutrient uptake, p. 288-307. In: Y. Kalpulnik and D.D. Douds, Jr. (eds.). Arbuscular mycorrhizas: Physiology and function. Kluwer Academic Publishers, Netherlands.

Gerdemann, J.W. and Nicolson T.H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235-244.

Gibson, D.J., and Hetrick, B.A.D. (1988) Topographic and fire effects on the composition and abundance of VA-mycorrhizal fungi on tallgrass prairie. Mycologia. 80: 433-451.

Giovannetti, M. and Mosse, B. (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Gupta, A.K., Shruti Chaturvedi and Sharma, A.K. (2009). Arbuscular mycorrhizal fungal diversity in some medicinal plants. Mycorrhiza News. 20(4): 10-13.

Hayman, D.S. (1982). Practical aspects of vesicular arbuscular mycorrhiza. Eds. Subba Rao N.S. In *Advances in Agricultural Microbiology.* Oxford and IBH publishers, New Delhi. Pp. 325-373.

Heckman, D.S., Dieser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L., and Hedges, S.B. (2008). Molecular evidence for the early colonization of land by fungi and plants. Science. 293: 1129-1133.

Hetrick, Daniels, B.A., and Bloom, J. (1983). Vesicular-arbuscular mycorrhizal fungi associated with native tall grass prairie and cultivated winter wheat. Canad. J. Bot., 61: 2140-2146.

Javaid Arshad, Tariq Riaz and Salik Nawaz Khan (2007). Mycorrhizal Status of *Narcissus papyraceus* Ker-Gawl. co-cultivated with *Cynodon dactylon* Pers. International Journal of Agriculture & Biology. 9(6): 901-904.

Koske, R.E. and Tiss1er, B. (1983). A convenient, permanent slide mounting medium. Newsletter Mycol. Soc. Am. 34(2): 59.

Liberta, A.E., and Anderson, R.C. (1986). Comparison of vesicular-arbuscular mycorrhiza species composition, spore abundance and inoculum potential in an Illinois prairie and adjacent agricultural sites. Bull. Torrey Bot. Club. 113: 178-182.

Marc Ducousso, Heriniaina Ramanankierana, Robin Duponnois, Raymond Rabevohitra, Laurent Randrihaspara, Manon Vincelette, Bernard Dreyfus and Yves Prin, 2008. Mycorrhizal status of native trees and shrubs from eastern Madagascar littoral forests with special emphasis on one new ectomycorrhizal endemic family, the Asteropeiaceae. New Phytologist. 178: 233-238.

Mosse, B. (1973). Advances in the study of vesicular arbuscular mycorrhizal. Annual Review of Phytopathology, 11: 171-196.

Peterson, R.L., Massicote, H.B., and Melville, L.H. (2004). Mycorrhizas: Anatomy and cell biology. NRC Research Press, Ottawa, Ont.

Philips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungus for rapid assessment of infection. Trans. Br. Mycol. Sci. 55: 158-161.

Piper, C.S. (1966). Soil and plant analysis. Adelaide University Press, Australia.

Qadri, R. (2004). Presence of endomycorrhiza and root nodules in *Samanea saman* (Jacq.) Mess. Hamdard Medicus. XLVII: 5-7.

Rajni Gupta and K.G. Mukerji, 1996. Studies on the vesicular arbuscular mycorrhizal associations in the root of common Delhi grasses. Phytomorphology. 46(2): 117-121.

Redecker, D., Kodner, R., and Graham, L.E. (2000). Glomalean fungi from the Ordovician. Science. 289: 1920-1921.

Renker, C., Blanke, V., and Buscot, F. (2005). Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. Environmental Pollution, 135: 255-266.

Sadiq Gorsi, M. (2002). Studies on Mycorrhizal Association in Some Medicinal Plants of Azad Jammu and Kashmir. Asian Journal of Plant Sciences, 1(4): 383-387.

Sampath Kumar, G. and Murugesh, S. (2002). Studies on the VAM fungi improves growth of some medicinal plants. Ad. Plant. Sci., 15(1): 43-46.

Schenck, N.C., and Perez (1990). Manual for the identification of VA Mycorrhizal fungi. INVAM, University of Florida, Gainesville, Fk, Pp: 245.