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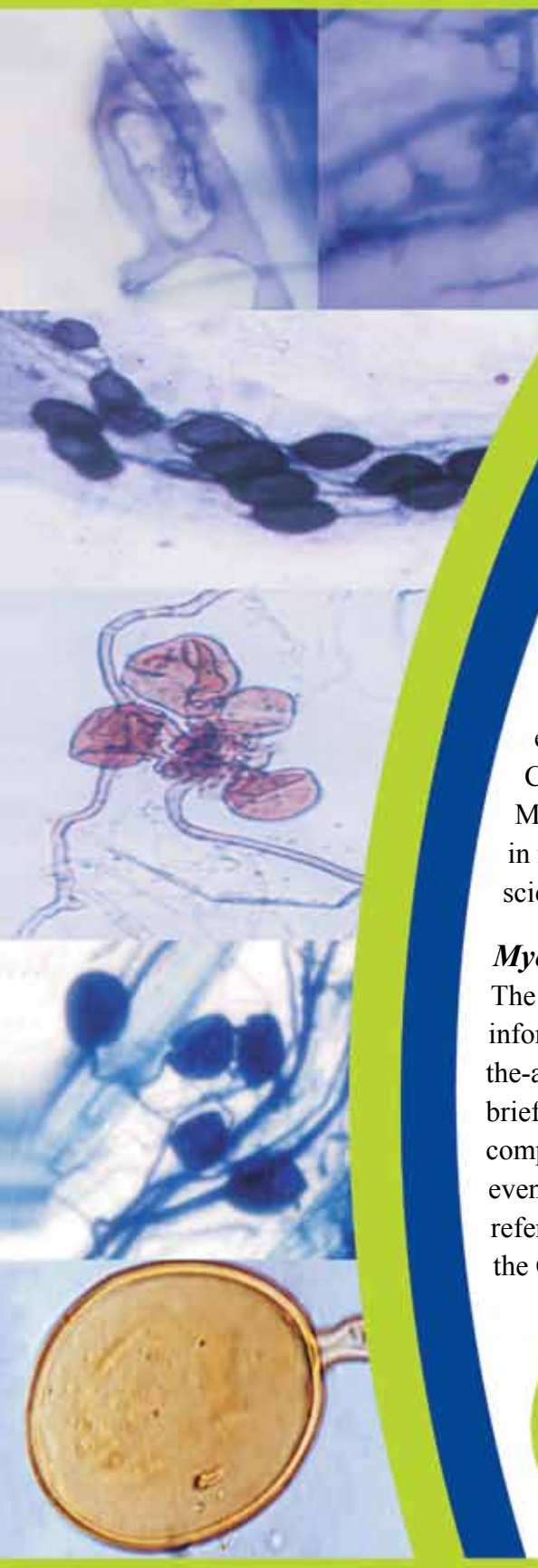

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TERI's Mycorrhiza Network is primarily responsible for establishing the MIC (Mycorrhiza Information Centre), the CMCC (Centre for Mycorrhiza Culture Collection), and publishing Mycorrhiza News. The Network helps scientists carry out research in mycorrhiza and promotes communication among mycorrhiza scientists.

Mycorrhiza News

The Mycorrhiza News provides a forum for dissemination of scientific information on mycorrhiza research and activities; publishes state-of-the-art papers from eminent scientists; notes on important breakthroughs; brief accounts of new approaches and techniques; publishes papers complied from its RIZA database; provides information on forthcoming events on mycorrhiza and related subjects; lists important research references published during the quarter; and highlights the activities of the CMCC.

Contents

Research finding papers

AM fungi in wild banana I: distribution and ecology*

Sharda W Khade#

Introduction

The Western Ghats, one of the 25 mega-diveristy 'hot spots of the world', embodies more than 4,000 of the country's endemic plant species distributed along wide range of geo-climatic conditions (Bhat 2002). The state of Goa lies in the heart of Western Ghats (Khade and Rodrigues 2002). The major portion of the slopes of Western Ghat belt falls in this region encompassing luxuriant forests with good diversity (Rao 1985). Three talukas of Goa *namely,* Sattari, Sanguem, and Canacona come under Western Ghat's belt exhibiting deciduous/semi-evergreen forest, abandoned lands, roadsides, low lying land tracks, cultivated plantations, and wild gardens (Bhat 2002).

Banana belonging to family Musaceae contains two genera -*namely, Musa* and *Ensete*. *Musa* is best divided into four sections one of which (*Emusa*) contains the great majority of the edible bananas (derived from *M. acuminata* and *M. balbisiana*) and the other three yield one important fibre (*Abaca*, from *M. textalis, Australimusa*), one minor group of edible cultivar and others are used as, vegetables and ornamentals (Khade1999).

At present, in the state of Goa, banana occupies an area of 1,875 ha with corresponding production of 10,650 tonnes which is sufficient to meet 40% of the requirement of the state. The balance 60% is met by import from neighbouring states (Khade 1999). The crop is grown in almost all parts of Goa but concentrated in talukas of Bicholim, Sattari, Sanguem, Ponda, Pernem, and Canacona. The varieties like Poovan *Musa* (AAB), *Raspali* (AAB), *Hazari* (AB), *Saldatt*i (AAB), and *Savarbondi* (ABB) etc., are grown, of which *Raspali, Hazari*, and *Saldatti* are commonly cultivated and are of economic importance (Khade 1999). Keri, at the foot hills of Chorlem Ghat is also known for extensive banana cultivation. Wild species of banana were observed for the first time in Chorlem Ghat during a survey on inventorization and documentation of plants from Western Ghat region of Goa. Following which survey was carried out to ascertain AM fungal association in wild banana from Chorlem Ghat region of Goa.

Materials and Methods

Root samples and the rhizoshpere soil samples of wild banana (Figure 1 B, C, D) were collected during the post monsoons (October 2001) and summer (March 2002), from Chorlem Ghat (Figure1A) region of Goa. Further, freshly collected roots of wild banana were washed in water, cleared with 10% KOH, acidified with 1N HCl and stained in 0.05% Trypan Blue in lactoglycerol (Phillips and Hayman 1970). Quantification of root colonization of AM fungi was carried out using slide method (Giovannetti and Mosse 1980). Spores of AM fungi were extracted from the rhizosphere using wet sieving and decanting method (Gerdemann and Nicolson 1963). Quantification of spore density of AM fungi was carried out using a method given by Gaur and Adholeya (1994). Diagnostic slides with spores were prepared using Polyvinyl Alcohol Lacto Glycerol

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This paper is a part of the series of a paper on wild banana. Part 2 and part 3 of the series have been already published previously.

(A)

(B)

(C)

(D)

Figure 1 (A)–(C) An overview of Chorlem Ghat; (B) - (D) Wild banana in its natural habitat

(PVLG) as mountant. Identification of AM fungi was carried out using manual of identification of the AM fungi (Schenck and Perez 1990). Taxonomic identification of spores was matched with the descriptions provided by International Collection of Vesicular AM fungi (http:// invam.caf.wvu.edu). Standard deviation was calculated for mean root colonization and mean spore density of AM fungi.

Results

The climatic conditions prevailing during the study period at site Chorlem and AM fungal status of wild banana is recorded in Table 2**.** The humid climatic conditions prevailing during October and March were favorable for conducting study on the AM fungi. This study recorded the presence of hyphal and vesicular colonization of AM fungi (Figure 2A)

Table 2 Arbuscular mycorrhizal association in wild banana

Plant species	Region	Sampling time	Rainfall (mm)	Temperature (Min ^{0}C)	Temperature (Max ^{0}C)	*Root Colonization (%)	*Spore density /100g soil	Species of AM fungi
Musa sp. (wild banana)	North Goa (Western Ghat- Chorlem)	October 2001	61.6	31.5	24.6	20 ± 0.07	100 ± 2.86	*Glomus fasiculatum*
		March 2002	–	32.9	25.9	10 ± 0.14	26± 1.48	*Glomus clarum* *Glomus goaensis* *Indiana bulbigoa*

* n= 5 ± 1SD

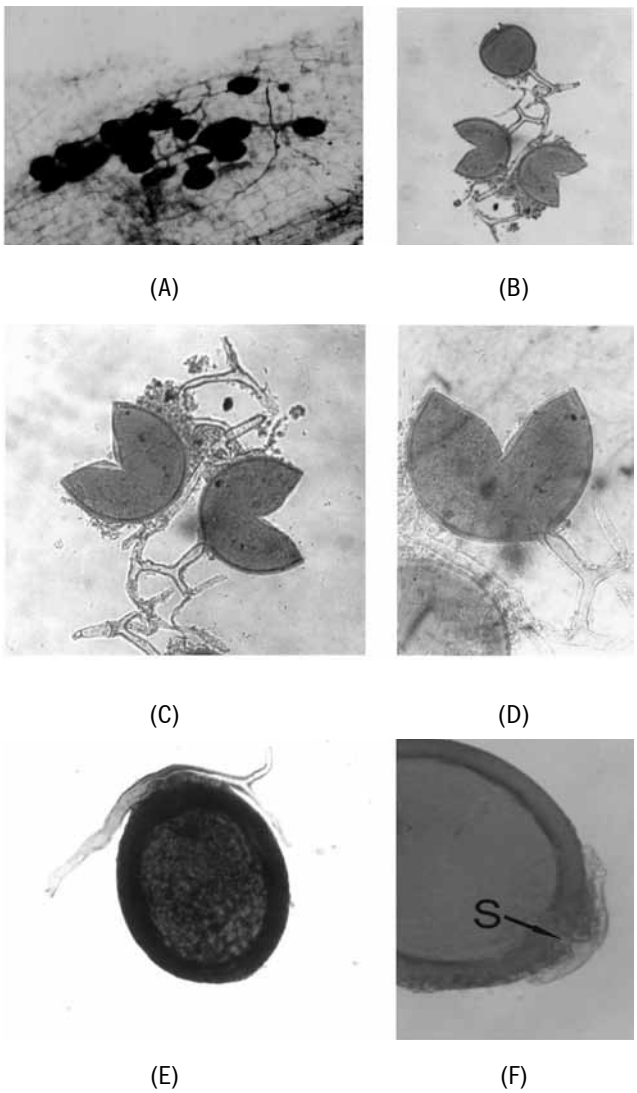

Figure 2 A) Hyphae and Vesicles stained in trypan blue (x 400).
B), C) Spores of *Glomus fasiculatum* occurring in clusters (fasicles) (x 400).
D) Crushed spore of *Glomus fasiculatum* showing the extension of spore wall into the hyphal attachment (x 1000).
E) Mature brown spore of *Glomus clarum* with hyphal attachment (x 400).
F) A portion of reddish brown spore of *Glomus clarum* showing bulging septum (S) at the point of attachment (x 400).

during post monsoon. With mild rain showers during October, the root colonization (20%) and spore density (100 spore/100g soil) of AM fungi was higher as compared to March where the root colonization (10%) and spore density (26 spore/100g soil) of AM fungi was low (Table 2). In contrast to the above findings, the number of species of AM fungi was lower in post monsoon (1 species) as compared to summer (3 species) in the absence of rain showers and comparatively higher temperatures. The study recorded the presence of *Glomus fasiculatum* (Thaxter) Gerd. & Trappe emend Walker & Koske (Figure 2 B, C, D) in October and recorded three species of AM fungi *namely*, *Glomus clarum* Nicol, and Schenck (Figure 2 E, F), *Glomus goaensis* Khade and *Indiana bulbigoa* Khade during March (Table 2).

Discussion

AM fungi forming symbiotic association with majority of land plants mainly comprises three phases of growth within the host root namely, hyphae, arbuscules, and vesicles. Apart from these, spores of some species develop within the roots and in most cases spores are formed outside the root on the runner hypahe (Khade and Adholeya, 2005). In the present study, intraradical hyphal and vesicular colonization was evident in epidermal and cortical root region of wild banana while the functional stage of AM fungi *namely*, dichotomously branched arbuscules was absent. The globose and ellipsoidal vesicles encountered in the present study represented genus *Glomus*. The AM status and diversity of AM fungi associated with cultivated *Musa* species in India is represented in Table 1.

In Kerala (India), the banana cultivar (*Musa sapientum* L) recorded 18.1% to 60.9% root colonization of AM fungi (Girija and Nair, 1988). In South Goa, banana cultivar (*Musa paradisiacal* L) recorded 66% root colonization and 20 spores/100g rhizosphere soil (Bukhari *et al.*, 2003) (Table 1). Similarly in North Goa, the banana cultivar *Saverbondi* (ABB) recorded 426 spores/100g rhizosphere soil in monsoon and 250 spores/100g rhizosphere soil post monsoon (Khade and Rodrigues, 2004) (Table 1). In

Table 1 Comparative account of AM status of cultivated banana variety in India

Area/ Region	Variety/ Cultivar	Sampling time	Root Colonization (%)	Spore density/100g soil	Species of AM fungi	Reference
Kerela	*Musa sapetium* L.					
	Padatti	–	26.6	–	–	Girija & Nair, 1988
	Red banana	–	57.7	–	–	
	Monthan	–	38.5	–	–	
	Pacha kappa	–	19	–	–	
	Rasthali	–	24.4	–	–	
	Chingan	–	18.1	–	–	
	Palayanthodan	–	60.9	–	–	
	Nendran	–	22.7	–	–	
Goa	*Musa paradisiaca* L.					
South Goa, Sanguem, (Quepem)			66 ± 6.9	20± 8	*Glomu macrocarpum, Acaulospora spinosa*	Bukhari *et al.* 2003
North Goa, Sattari, (Valpoi)	*Savarbondi*	May 1998		426 ± 21.45	*Acaulospora foveata Acaulospora nicolsonii Acaulospora scrobiculata Glomus claroideum Glomus monosporum Glomus gerdmanii Glomus clavisporum*	Khade & Rodrigues, 2004
		August 1998		384 ± 29.40	*Acaulospora foveata Acaulospora scrobiculata Glomus claroideum Glomus monosporum Glomus clavispora*	
		November 1998		250 ± 20.22	*Acaulospora scrobiculata Glomus claroideum Glomus monosporu m Glomus clavisporum*	

contrast to the earlier findings on banana cultivars, present study recorded lower root colonization and spore density of AM fungi in wild banana and that these parameters decline from post monsoon to the onset of summer in Goa, India.

Further, Bukhari *et al.*, (2003) and Khade and Rodrigues (2004) reported two genera of AM fungi (*Acaulospora* and *Glomus*) from rhizosphere of banana cultivar in South Goa and North Goa (Table 1). Similarly, the present study on wild banana reported the presence of two genera of AM fungi - namely, *Glomus and Indiana. The study supports the findings of* Khade and Rodrigues (2004), that maximum number of species of AM fungi are present in summer, as compared to post monsoon and in the present study species number actually increased from post monsoon to onset of summer in Goa, India.

Conclusions

Wild banana documented in the present study occurs in rocky areas near earth cutting along the road sides and therefore an attempt was made to report the gene pool of AM fungi associated with it in the Western Ghat region (Chorlem) of Goa, India. Though the role of AM fungi in agro-ecosystem is enhancing

plant growth, yield, and reducing the hazardous effect of chemical fertilizers, but in natural ecosystem like Western Ghat, AM fungi (hyphae) acts as an extension and increases the root surface area for uptake of water and nutrients, thus enabling survival of plant species like wild banana in inhospitable sites.

Acknowledgements

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Diversity of arbuscular mycorrhizal fungi associated with the rhizosphere of some existing plants in iron ore mines of Chhattisgarh, India

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Introduction

'Mycorrhizas are symbiotic associations essential for one or the both partners, between a fungus (specialized for life in soils and plants) and a root (or other substrate-contacting organ) of a living plant, that is primarily responsible for nutrient transfer. Mycorrhizas occur in a specialized plant organ where intimate contact results from synchronized plant-fungus development.'*Arbuscular mycorrhizae* (AM) are generally aseptate fungi classified under *Glomeromycota*. Arbuscular mycorrhizal fungi(AMF) have a loose external mycelium extending the hyphae into the soil beyond the depletion zone from which penetration of the host root cells takes place(Baruah and Borthakur, 1997; Jacobsen, 2004).

Mining land sites are generally known to be nutrient-poor and contain soils that are in direct need of stabilization to prevent erosion. Marked by the beginning works of J R Schramm, mine reclamation practices have included the use of mycorrhizal inocula to establish successful plant communities on mine sites (Danielson, 1985). Mycorrhizae benefit the vegetation by increasing a plant's ability to survive in a nutrient-poor, water-deficient environment in undisturbed ecosystems, and mycorrhizal relationships occur naturally. Mined sites, however, are chemically, physically, and biologically, altered and often lack the necessary quantity of mycorrhizal fungi to sustain a tolerant plant community (Norland, 1993). Several types of mycorrhizal inocula are currently in use and will be examined according to their practicality and economy to site specificity (Sturges Susan, 1997). The survey was conducted in mine sites known to harbor the plants present in the area. Generally *Dulbergia sisso, Adina cardifolia, Tactona grandis, Gmelina arborea, Shorea robusta, Diospyros melanoxylon, Delonix regia, Dendrocalamus strictus, Terminalia tomentosa, Pterocarpus marsupium, Cleistanthus collinus,Croton bonplandianum, Madhuca indica, Anogeissus latifolia,* and others.

Materials and Methods

Study and Geology of area

The present study was carried out of two iron ore mine areas of Chhattisgarh, the name are Rajhara mines and Ari Dongari mines. Rajhara iron mine is situated at lattitude 20°35' N and log 81°05' E, to south of Dalli-Rajhara taluk, Durg. It is located at about 83 KM south of Durg district, and comes under the south eastern section of the Indian Railways. The Rajhara mine has been mined systematically for iron over four decades. Rajhara mines occur in the semi-arid region in the southern plains of Chhattisgarh. The region is characterized by annual rainfall of 1071.16 mm. The day temperature during the year is around 25-45°C. Ari Dongari iron mines is of the 4.8 km long ridge extending in a N-S direction west of the village Kachche, which is located at about 26 km from the Rajhara mines on the fair weather road between Balod and Narainpur of Kanker district. The region is characterized by annual rainfall of 1190 mm, the day temperature during the year is around 20-40°C (Figure1).(Department of mineral resources, Government of Chhattisgarh).

Collection of Soil Samples and Root

Soil samples were collected from the three regions of iron ore mines; unmined area, amined area, and dumped area (topsoil before mining). The sampling location sites were randomized after site facing. The soil sample collected from ten different locations of all three areas. The core method will be used to take samples from 30cm depth of the selected site soil samples.

Root samples were collected from each mine, of few plants like *Dulbergia sissoo, Cleistanthus collinus, Croton bonplandianum, Madhuca indica, Anogeissus latifolia*. Each sample had five replicates. Fine roots of mature trees were excavated after tracing larger roots from the stem collar of plants. They were packed in polythene bags, along with the roots, and kept in a refrigerator in the laboratory until they were analysed.

Physical Properties of Soil

Three soils were checked for their physical parameters like temperature, pH, total dissolve solids, and conductivity (Table1). (Deluxe soil and water analysis kit model 172, company EI).

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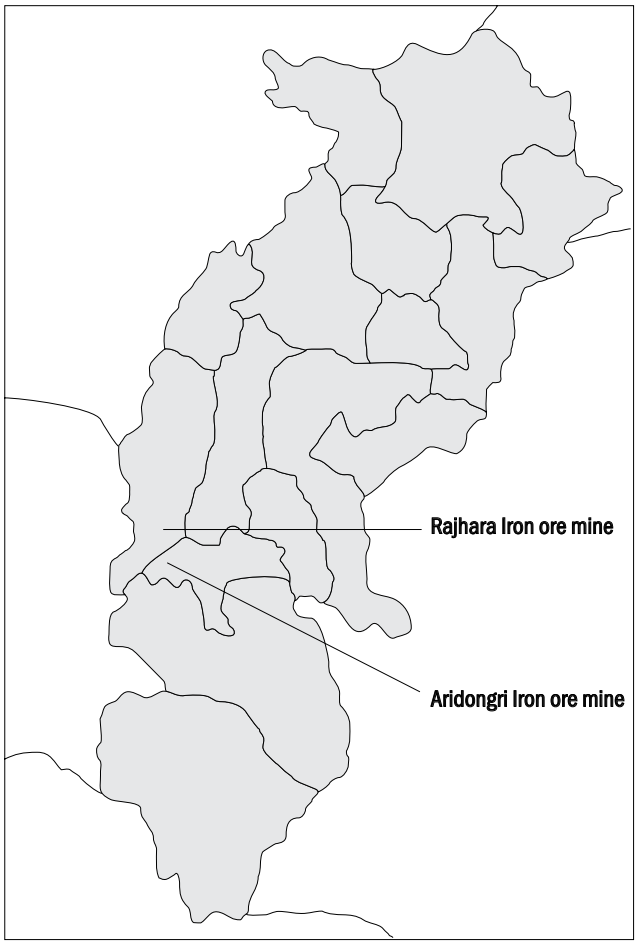

Figure 1 Map of Chhattisgarh showing location of Rajhara mines and Ari Dongri iron ore mines.

Mycorrhizal Percentage Infection

Trypan blue method (Phillips and Hayman 1970) was used for the determination of the intensity of root percentage infection. The method was also suitable for the determination of the root percentage infection of mycorrhiza, rate of colonization, and comparation of structures. The percentage root infection was calculated using the following formula:

$$\% \text{ root infection} = \frac{\text{Total mumber of root segments infected}}{\text{Total number of root segments examined}}$$

Spore Extraction and Identification

Spore extraction from the soil was carried out using wet sieving and decanting technique(Gerdemann and Nicolson 1963). Sieves size ranging from 500 μm to 75 μm were used to separate the spores from the soil samples. The sieves spore were placed in filter paper and observed with a stereo-microscope under reflected light. The spore density was expressed as the total number of spores occurring in 100 gm of soil. The spores were then observed for distinguishing morphological characters such as size, shape, and wall characteristics under a compound microscope (model: Labomed CXL PLUS). The spores were identified according to the manual of identification of vesicular-arbuscular mycorrhizal (AM) fungi by Schenck and Perez (1990), Morton and Benny (1990), and Mukerjee (1994). The INVAM worksheet for spore characterization was used for diagnosing the spores.

Result and Discussion

Pioneer research work on AM in mine reclamation practices were conducted by Danielson (1985), Norland (1993) and Sturges Susan (1997), who reported that the plants existing in mines area were found associated with arbuscular endotrophic mycorrhiza. The mycelia were found extended in the cells of the primary cortex, except in the root tips. Similarly, *Gigaspora*, *Glomus*, *Sclerocystis* were identified in the Rajhara iron ore mines soil and only the two order *Gigaspora* and *Glomus* werefound in Ari Dongari iron ore mine's soils.

Six soil samples were collected from three different zones of mine unmined and old dumped zone of Rajhara, and Ari-dongari iron ore mines area.

Mycorrhizal population of Rajhara iron ore mines has been presented in Figure 2. Spore population/ 100 gm soil was calculated. The spore population ranged from 0 to 03 spore/g of soil. Soils of unmined zone are harboured with highest spore population, the soils of dumped zone having less spore population, and the soil of mining zone is negligible spore

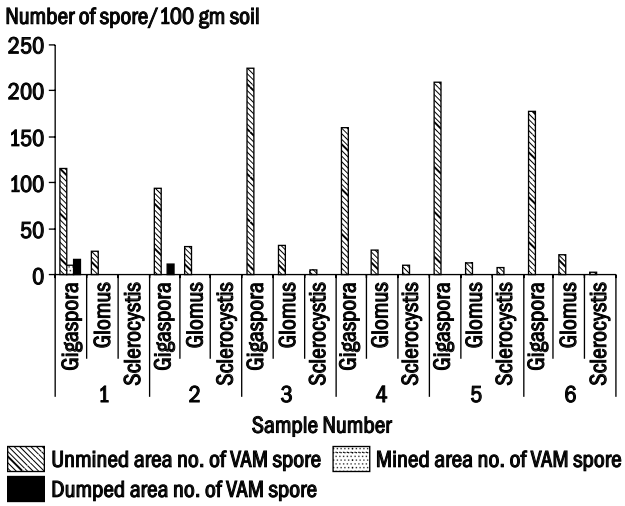

Figure 2 Number of arbuscular mycorrhizal fungi spores per 100 gm of soil (Rajhara iron ore mines)

Table1 Physical properties of soil Parameters

	Soil samples of Rajhara iron ore mines (mean value)			Soil samples of Aridongri iron ore mines (mean value)		
	Unmined zone	Mined zone	Dumped zone	Unmined zone	Mined zone	Dumped zone
Temperature (°C)	30.50	30.50	30.50	30.50	30.50	30.50
pH	6.55	7.28	7.48	6.32	7.26	7.66
Conductivity (ms/cm)	0.05	0.20	0.09	0.05	0.26	0.14
TDS(ppm)	31.67	141.00	39.33	37.67	147.00	43.00

population. Overall, three genus of AMF were detected from the study areas. The genus *Gigaspora* was found to be dominating in the unmined zone. *Glomus* and *Sclerocystis* are the secondary, and tertiary, moderately distributed genus in the unmined zone and very less in mined area.

Mycorrhizal population of Ari-dongari iron ore mines with their order has been presented in Figure 3. Spore population of 100 gm soil was calculated. The spore population ranged from 0 to 0.5 spore/g of soil. Soils of unmined zone are harboured with highest spore population, the soils of dumped zone having less spore population, and the soil of mining zone is negligible spore population. Overall, two genus of AMF was detected from the study areas. The genus *Glomus* was found to be dominating in the unmined zone. *Gigaspora* is the second moderately distributed genus in the unmined zone and very negligible number in few sample of dumped and mined zone are also absent in few sample of soils.

Physical parameter of soil samples are temperature, pH, total dissolve solids, and conductivity tested from randomly three sample of each zone. The result has been presented in Table1. The pH of dumped zone soil is higher than mined zone soil. Soil testing helps us to know the nutritional status of the soil, to assess the profitability of applying a particular nutrient. If soil pH is very high then there is no use of adding fertilizer for revegetation. The conductivity (ms/cm) and total dissolve solid (ppm) are more in soil collected from mined zone, as compared to soil collected from dumped zone. The excessive conductivity and total dissolve solid can also lower the efficiency of applied fertilizer for revegetation as well as mycorrhizal spore germination.

Percentage root infection of arbuscular mycorrhiza fungi of some existing plant root is given Figure 4. The percentage root infection by arbuscular mycorrhiza was found to be significantly influenced by physio-chemical properties of mines zone soil.

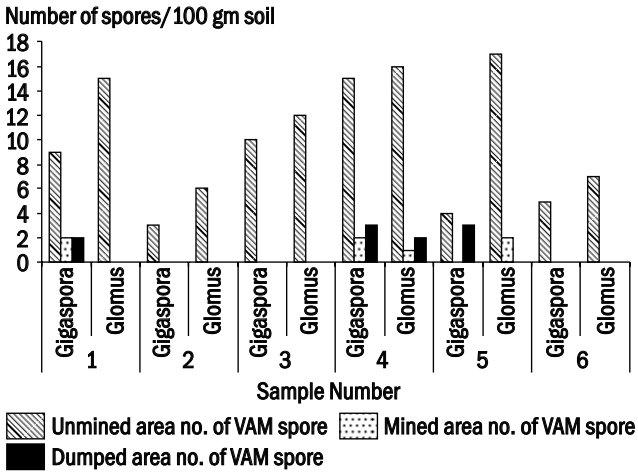

Figure 3 Number of arbuscular mycorrhizal fungi spores per 100 gm. of soil (Ari-Dongari iron ore mines)

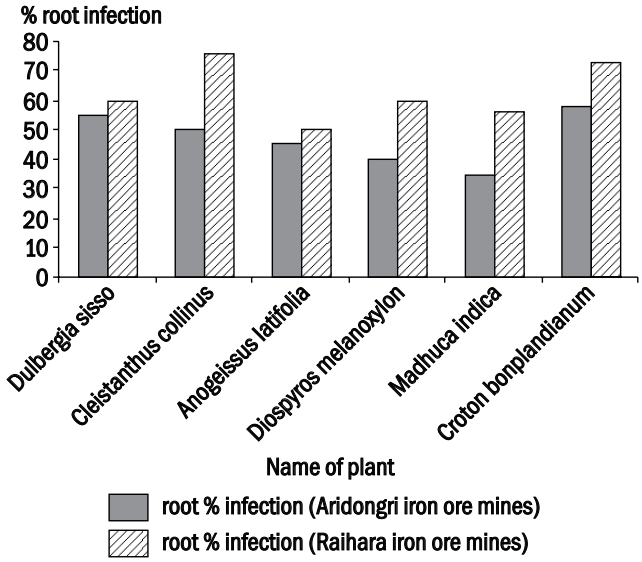

Figure 4 Root percentage infection of mycorrhizal

Conclusion

Following are the important experimental findings of the prevent investigation

- The excessive conductivity and total dissolve solid can also lower the efficiency of applied fertilizer for revegetation, as well as mycorrhiza spore germination.
- Soil testing helps us to know the nutritional status of the soil to assess the profitability of applying a particular nutrient.
- If soil pH is very high there is no use of adding fertilizer for revegetation.

Chhattisgarh is geologically one of the most important terrains in the Indian shield, comprising litho logical sequence ranging in age from archaeanthe to recent. Mineral resources and mining activities from major contribution in industrial and economic development of an area. However, indiscriminate mining of the non-renewable resources not only depletes the natural resources but also involves inevitable disturbances to an ecological set up. Continued advancement in industrialization and the increasing demand for mineral resources have led to a spurd in mining activity, causing imbalance in ecological equilibrium, followed by many environmental hazards.

The restoration of site is difficult if scientifically not attended well in time. The upper surface of the soil is very active and is full of micro-organisms. They develop a particular type of symbiotic relationship which is unique to the type of soil and environmental conditions prevailing in the area, and the stabilization of such symbiotic relationship takes long time to develop mycorrhiza with plant roots. This association helps the plant to establish on sites, such as saline, alkaline, eroded, or distributed due to human activities. The present investigation is proposed with an objective to find out suitable host VAM associations in dominant plant species in selected mines of Chhattisgarh.

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Non–target effect of two fungicides on *Glomus mosseae* colonization of chilli roots

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Introduction

As fungicides either inhibit or kill fungi, they are expected to influence the population and root colonization by Arbuscular Mycorrhizae (AM) fungi. Since AM benefit plants variously by enhancing their nutrient and water status and/or increasing root resistance to soil-borne pathogens, such benefits may be reduced if the AM colonization is hampered. Detrimental effects of several fungicides on AM fungi have been reported previously (Meng, 1982; Kough et al., 1987; Dodd and Jeffries, 1989; Kjoller and Rosendahl, 2000; Chiocchio et al., 2000). The activities of Alkaline Phosphatase (ALP) (Thingstrup and Rosendahl, 1994; Kjoller and Rosendahl, 2000), and Succinate Dehydrogenase (SDH) (Kough et al., 1987; Kling and Jakobsen, 1997) in both the external and internal fungal hyphae have been used as criteria for rapid assessment of the effect of fungicides. These enzymes are important because they indicate active fungal metabolism. SDH, a mitochondrial enzyme, is considered an indicator of viability of mycorrhiza (Vierheilig & Ocampo, 1989, Guillemin et al., 1995) while ALP activity, located within the phosphate-accumulating vacuoles of AM hyphae (Gianinazziet al., 1979), has been proposed as a physiological marker for analyzing its efficiency (Tisserant et al., 1993).

Mancozeb, a polymeric dithiocarbamate protectant, and the systemic organ-phosphorus Kitazin are commonly used by local farmers in the management of foliar diseases of chilli. While the non-target effect of the contact fungicide in other crops has been reported, similar effect of systemic Kitazin has not been reported earlier. Hence, one of our objectives was to evaluate the non-target effect these fungicides on mycorrhizal colonization in chilli roots and to record SDH and ALP activity of the fungal structures.

Imaging technique, like laser scanning confocal microscopy (LSCM) using different fluorochromes, has been effectively used to study AM and ECM structures (Czymmek et al., 1994; Kobayashi et al., 1994; Schelkle et al., 1996; Dickson and Kolesik, 1999). Kumar et al., (2008), successfully used and reported the suitability of trypan blue, a non-vital stain, as a dye in fluorescence microscopy. Following their work, we decided to study and compare the LSCM images of fungicide treated and untreated roots, using trypan blue as a fluorochrome.

Material and Methods

2.1. Experimental set-up

Seeds of *Capsicum annum* (chilli) were surface sterilized, sown into plastic pots (6" height) containing sterilized alluvial soil. *Glomusmosseae*, isolated from the same soil, was mixed with the sterilized soil, seven days before sowing (@ 25g of infected roots pieces of *Zea mays* L + external mycelium and spores/kg of soil). Mancozeb and Kitazin were applied as a single application to the foliage of two-month-old plants with a hand-sprayer, without covering the soil in the pot, at the recommended field–rate concentration of application. A non-fungicidal treatment was set up as control. Each treatment was replicated thrice. The plants were grown in the greenhouse under controlled conditions.

Harvest and Analysis

Root samples were collected randomly from each treatment, per replication, three and fourteen days after fungicidal application. The roots were washed separately in cold tap water, blotted dry, cut into 1 cm segments, and suspended in cold tap water. Each lot was divided into three portions of twenty fragments each. One fraction was cleared by 10% KOH and stained for fungal structures with trypan blue (TB) (Phillips and Hayman 1970). The second was stained for fungal ALP activity (Tisserantet al., 1993) and the third for SDH activity (Macdonald & Lewis, 1978). Established protocols were used for scoring the percentage of colonization by *G.*mosseae, ALP and SDH activity in the root fragments. MYCOCALC online computer program was used to calculate the intensity of the mycorrhizal colonization in the root system (M %) and arbuscule abundance in the root system (A %).

2.3. LSCM study

Root samples for LSCM study were harvested, fourteen days after fungicidal application, from treated and corresponding control plants of the same age. Roots were cleared and stained as mentioned earlier (Phillips and Hayman 1970). TB dye was freshly

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prepared and served as a flourochrome. Laser scanned and bright-field images of the root segments were obtained on a Leica Confocal System-TCS SP2. Emission was observed through high magnification oil-emersion objective using laser beams with 488 and 543 nm excitation wavelengths and a 585 nm Long Pass (LP) emission filter.

2.4. Statistical analysis

Percentage data were arcsine-transformed before analysis and subjected to one-way Analysis of Variance (ANOVA). The treatment means were separated by a Student-Newman-Keul's test.

3. Results

3.1. Effect on AM fungus root colonization and arbuscle formation

Application of both fungicides significantly reduced total mycorrhization in the root system (M %) of chilli plants as compared to the percent colonization in the untreated ones (Table 1). In Kitazin, percentage root colonization further decreased from three to fourteen days after application, while in Mancozeb treatment there was no further reduction in mycorrhization of roots recorded on the fourteenth day after the initial reduction. Arbuscle formation in the roots was significantly reduced by fungicide treatment, the effect being greater in Kitazin than in Mancozeb(Table 1).

3.2. Effect on SDH and ALP activity

In Kitazin treatment, activities of SDH and ALP in the internal hyphae were significantly depressed after three days of fungicide application and a further decrease was recorded in root samples collected fourteen days after application while Mancozeb appeared to have no effect on either of the enzyme activities within the internal hyphae, irrespective of the days after application(Table 1).

While some SDH and ALP activity was recorded in the arbuscles in untreated roots, neither SDH nor ALP- active arbuscles were observed in either of the fungicide treated roots collected three days after application. However, in Mancozeb treated roots, trace SDH and ALP activity was recorded in the arbuscles, fourteen days after application.

3.3. LSCM image analysis

LSCM images (Figure 1) of roots, collected fourteen days after fungicide application, showed bright green fluorescing structures of *G mosseae* only in TB-stained control and Mancozeb treatment. In roots treated with Kitazin, most of the fungal structures did not fluoresce. The fungal structures were clearly visible in the corresponding bright-field images (Figure 1).

4. Discussion

Fungicides have been variously reported to either harmfully effect or have no effect on AM fungi. Tubulin-biosynthesis inhibitor benomyl and its breakdown product carbendazim, the heterocyclic phthalimidecaptan, sterol inhibitors fenpropimorph, and propioconazole are reported to inhibit the internal and external hyphae, hyphal ALP-activity, hyphae, and arbuscle - SDH activity and P uptake at concentrations lower or higher than the recommended field application dosages (Trappe et al.,1984; Kough et al., 1987; Kling and Jakobsen, 1997; Schweiger and Jakobsen,1998; Kjollerand Rosendahl, 2000). The inhibitory effects of some dithiocarbamates (Ziram and Mancozeb) on root colonization by the fungus and on spore production and germination have also been noted in literature (Sreenivasa and Bagyaraj, 1989; Vijayalakshmi 1993; Giovannetti et al., 2006; Saleh and Al-Garni, 2006). However, fungicides, like the broad-spectrum, strobilurins like azoxystrobin and kresoxim-methyl were not found to affect mycorrhization of crop plants at the recommended dosages (Diedhiou et al., 2004).

Table 1 Mycorrhization, arbuscle formation, SDH and ALP activity in the chilli root system treated with Kitazin and Mancozeb.

	Mycorrhizal colonization in the root system(M%)						Arbuscule abundance in the root system (A%)					
Treatment	Internal hyphae(%)		SDH-active fraction (%)		ALP-active fraction (%)		Arbuscles(%)		SDH-active fraction (%)		ALP-active fraction (%)	
	Days after fungicide application											
	3	14	3	14	3	14	3	14	3	14	3	14
Control	55.99*a	67.33a	44.54a	48.59a	39.57a	45.81a	23.24a	27.37a	6.56	12.95a	5.70	12.94a
Kitazin	27.25b	22.12b	18.54b	16.56b	15.29b	14.39b	2.12b	0	0	0	0	0
Mancozeb	29.02b	30.30c	24.33c	24.77c	21.31c	23.15c	12.86c	5.71b	0	4.21b	0	2.92b

*Each value is the mean of three replications. Data followed by the same letters within each column were not significantly different (P<0.05, Student Newman- Keuls' test). All percentage values were arc sin transformed before statistical analysis.

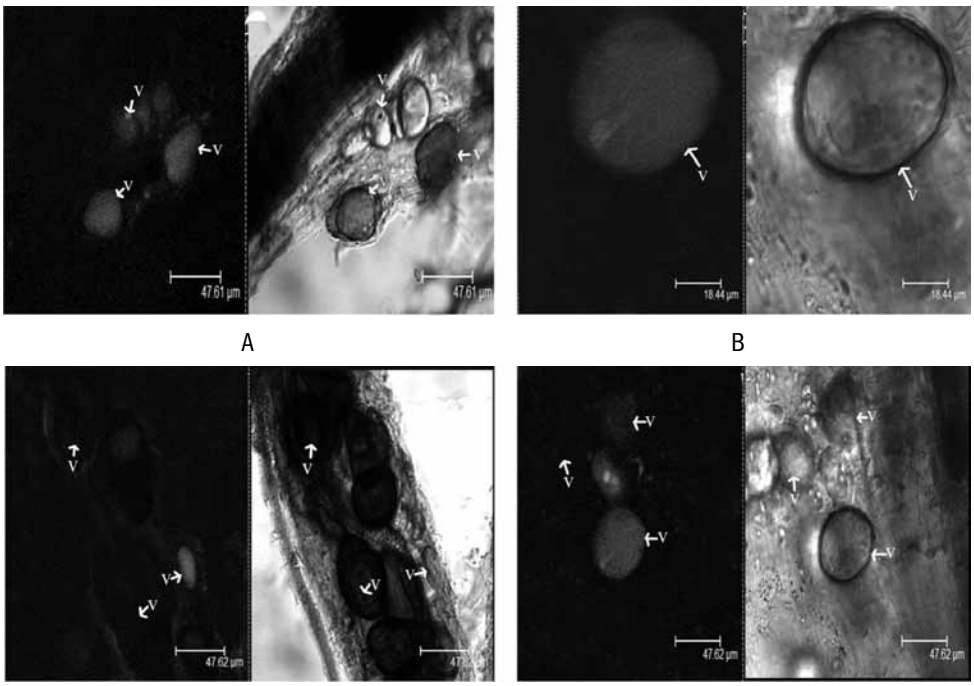

Figure 1 LSCM and bright-field images of chilli roots, fourteen days after fungicide application, showing vesicles (v) of *G Mossae*. Untreated (A and B), Kitazin-treated (C) and Mancozeb-treated (D).

In the present experiment, both fungicides were found to reduce root colonization and arbuscle formation by *G* mosseae, to statistically significant degrees at the recommended field rates of application. These results were in agreement with some of the earlier findings mostly on the effect of mancozeb. SDH and ALP activity in the hyphae appeared to be more sensitive to the systemic than the contact fungicide. However, the apparent reduction in the percentage of SDH-ALP active hyphae in the latter was probably due to the overall reduced fungal colonization. No enzyme activity was recorded in the arbuscles in Kitazin treatment but in Mancozeb treated roots, the effect of the fungicide appeared to wear off with time and low enzyme activity was recorded in the arbuscles after fourteen days.

Assuming that chitin, a major constituent of fungal cell wall, is the chief component stained by TB (Vierheiliget al., 2001), it could be presumed that fluorescence or non-fluorescence of the mycorrhizal structures could be due to the action of the fungicide directly on chitin cell wall of the fungus. This would cause the differential staining and hence the fungal structures would fluoresce depending on the amount of stain taken up by the cell wall.

The suggested mode of action of Kitazin is inhibition of chitin biosynthesis by inhibiting the incorporation of methioninemethyl-14C into phospholipids, especially phosphatidylcholine (Akatsukao et al., 1977). Treatment with Kitazin, might have either reduced the chitin content and consequently the thickness of the cell wall or may have resulted in changes in the chitin composition of the walls which affected their capacity to take up the dye.

Mancozeb,an ethylenebisdithiocarbamate (EBDC) group of non-systemic (surface acting) fungicide, acts by being metabolised to an isothiocyanate radical which inactivates the sulphydryl groups in amino acids contained within individual fungal cells and disrupts lipid metabolism. The overall effect may reduce the infestation and arbuscle formation by the mycorrhizal fungus but since it did not affect the cell wall directly, it did not make any impact on the TB staining of the fungal structure. Therefore, fluorescence was observed.

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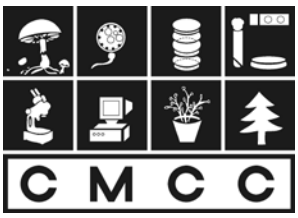

Centre for Mycorrhizal Culture Collection

Grasses: a trusted model plant for Mycorrhiza study

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In this edition, we would like to communicate to our readers the significance and role of the appropriate selection of a host plant towards successful symbiosis amidst the plant and Mycorrhiza. As we all know, the occurrence of this mutualism dates back to 400 million years. Barring a few families in the plant Kingdom, almost all types of flora choose to get involved in this symbiosis due to the enormous benefits that are imparted to the plant by this miracle fungus. The major outcome of this plant–fungus association involves the translocation of carbon from the plant to the fungus and also enhances uptake and transport of soil nutrients, primarily phosphorus, to the plant via the fungus. Other potential benefits of fungal colonization to host plants include improved uptake of poorly mobile nutrients such as zinc and copper, improved plant water relations, and reduced pathogenic infections.

Why do we need to think about the host at this point of time? The unpretentious answer would be, "as a collection bank, we are in hunt of a host that would be a Wonder Wand and can reproduce oodles of spores/ inoculum from the single spore that we use to develop pure culture". In one of our last editions, we had taught the art of raising monosporals. The major prerequisite for being a suitable host for inoculum production would be, primarily, the capability of rapidly being colonized and furthermore the root architecture of the plant should be such, that it has fine roots with good biomass. In order to search for one, we decided to review literature and find out the most favourite host on which researchers have done most of their Mycorrhizal studies. To our amazement we found that quite a large number of research articles rivet around plants belonging to Graminoids, more commonly known as grasses.

Grasses are monocotyledonous, usually herbaceous plants with narrow leaves growing from the base. They are conventionally categorized into three types. The first category includes the 'true grasses' of the Poaceae (or Gramineae) family, the second refer to the sedges (Cyperaceae), and the third is the rushes (Juncaceae). The true grasses include cereals, bamboo, and the grasses of the lawns (turf) and grasslands. Sedges include many wild marsh and grassland plants, and some cultivated ones such as water chestnut (Eleocharis dulcis) and papyrus sedge (Cyperus papyrus). After reviewing deeper, we comprehended that on one end the true grasses are considered to be an exceptional host for Mycorrhiza, and on the other hand both sedges as well as rushes are found to be incapable of having any symbiosis with Mycorrhiza. Hence, the statement that all grasses can be mycorrhized would be highly incorrect and deleterious.

Grasses are primarily divided into two physiological groups—C3 and C4—depending upon the photosynthetic pathway they follow during carbon fixation. The C4 grasses are considered to be warm season grasses and grow in atmospheres which are low in carbon dioxide, e.g., corn, Sudan grass, pearl millet, etc. Similarly, C3 grasses are referred to as 'cool-season' grasses, e.g., wheat, rye, oat, lolium, etc. Certain studies done on the tallgrass of prairie plants have demonstrated that all grass types belonging to the Poaceae get colonized in the presence of mycorrhizya but their dependence on mycorrhizal symbiosis for nutrient acquisition and growth differs. The warm-season, grasses C4 are extremely dependent (obligate mycotrophs) on mycorrhiza symbiosis, whereas cool-season grasses C3 are significantly less dependent on the symbiosis (facultative mycotrophs) (Kitt, and Wilson, 1988;, Wilson, and Todd, 1990). Hence, we infer that it may be advisable to select the warm season grasses for maintaining cultures for inoculum production.

While trying to analyse the different grass varieties that have been used for taking up mycorrhizal studies, we found that a lot of research has been done on Bermuda grass (*Cynodon dactylon* L). It is an important turfgrass widely used in playgrounds and greenbelt areas. It has been found that the coarse root of these grasses show good colonization with correspondingly high plant performance in terms of grass establishment, root growth, fertilizer utilization, cover percentage, drought, nematode, and disease resistance (Jin Wu et

al., 2011). A series of grasses that include *Dasyochloa pulchella*, *Bouteloua barbata*, *Chloris virgata*, *Scleropogon brevifolius*, *Pleuraphis mutica*, *Trichloris crinite*, *Prosopis glandulosa* var. *torreyana*, *Flourensia cernua*, *Lippia graveolens*, and *Larrea tridentate* were used in a study to prove a hypothesis that plant species are more responsive to mycorrhiza in late than in early succession stages (Fabiana Pezzani et al., 2006). We would not elaborate on this hypothesis, but mention here that the grasses mentioned were all of the warm-season variety.

One of the areas where there has been some very extensive study on the interaction of Mycorrhiza and the tall grasses is the Prairie region. A microcosms was created using different species of grasses *Andropogon gerardii* (C4), Sorghastrum *nutans (*C4), *Koeleria pyramidata* (C3), and *Poa pratensis* (C3) to investigate the influence of arbuscular mycorrhizal fungi on plant species composition, relative abundances, and diversity (Wilson and Hartnett, 1997). On similar grounds, another study was conducted in which the effects of five different host plant species *Poa pratensis L, Sporobolus heterolepis, Panicum virgatum L, Baptisia bracteata Muhl*, and *Solidago missouriensis* were analysed on spore communities of AM fungi in tallgrass of prairie. Spore abundances and species composition of fungal communities of soil samples collected from patches within tallgrass prairie were significantly influenced by the host plant species that dominated the patch. The AM fungal spore community associated with *B bracteata* showed the highest species diversity and the fungi associated with *Pa virgatum* showed the lowest diversity. These results strongly suggest that AM fungi show some degree of host-specificity and are not randomly distributed (AH Eom et al., 2000). Another arena where a lot of research has been done is the effect of Mycorrhiza on pathogen. Grasses like *vulpia ciliiata spp ambigua* has been inoculated with *fusarium oxysporum* and it was found that AMF alters the root infecting mycoflora. In one of the articles about impact of host species on the culture of arbuscular fungi mentioned in the INVAM site, it was concluded that widely used tropical hosts plants for AMF studies included, *Stylosanthes* species, *bahia grass (Paspalum notatum)*, and *kudzu (Pueraria phaseoloides)*. Under conditions of low-light intensity and cooler temperatures, *Plantago lanceolata* appears to be a highly suited host. *Andropogon gerardii* (big bluestem), a C4 plant is also widely accepted since it is very similar to Sudan grass and has high mycorrhizal dependency.

This short review of literature proves that the success story of symbiosis between grasses and mycorrhiza is infinite. Trying to enlist all the grasses that have been used by different researchers till date in doing Mycorrhizal research is a Herculean task. But, we would like to conclude that grasses as host recommendation should be done after evaluating every parameter which is responsible for its growth since results and practices are not pertinent to every circumstance. However, we personally always prefer C4 grasses over C3.

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http://invam.caf.wvu.edu/otherinfo/articles/host.htm

Recent references

The latest additions to the network's database on mycorrhiza are published here for the members' information. The list consists of papers from the following journals.

- *Agriculture, Ecosystems & Environment*
- *Applied Clay Science*
- *Applied Soil Ecology*
- *Current Opinion in Plant Biology*
- *Environmental and Experimental Botany*
- *European Journal of Soil Biology*
- *Fungal Ecology*
- *Fungal Genetics and Biology*
- *Industrial Crops and Products*
- *Journal of Hazardous Materials*
- *Journal of Plant Physiology*
- *Pedobiologia*
- *Pedosphere*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*

Name of the author(s) and year of publication	Title of the article, name of the journal, volume number, issue number, page numbers (address of the first author or of the corresponding author, marked with an asterisk)
Abbaspour H, Saeidi-Sar S, Afshari H, Abdel-Wahhab M A. 2012	**Tolerance of Mycorrhiza infected Pistachio (*Pistacia vera* L) seedling to drought stress under glasshouse conditions.** *Journal of Plant Physiology* **169**(7): 704–709 [Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Iran]
Angela Cicatelli, Guido Lingua, Valeria Todeschini, Stefania Biondi, Patrizia Torrigiani, Stefano Castiglione. 2012	**Arbuscular mycorrhizal fungi modulate the leaf transcriptome of a Populus alba L clone grown on a zinc and copper-contaminated soil.** *Environmental and Experimental Botany* **75**: 25–35 [Dipartimento di Chimica, Università di Salerno, Stecca 8, Via Ponte don Melillo, 84084 Fisciano (SA), Italy]
Arocena J M, Velde B, Robertson S J. 2012	**Weathering of biotite in the presence of arbuscular mycorrhizae in selected agricultural crops.** *Applied Clay Science* **64**: 12–17 [University of Northern British Columbia, Prince George, BC, Canada V2N4Z9]
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