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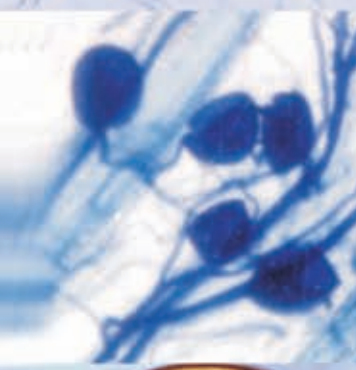
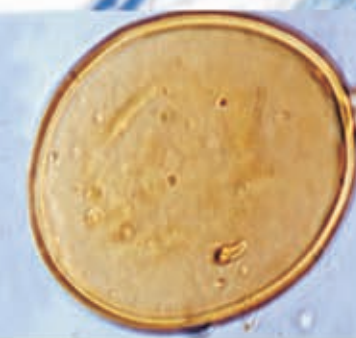

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Mycorrhiza News

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

Microbial succession and restoration of degraded ecosystem under different tree cover

O P Chaubey, Priyanka Bohre*, Jamaluddin**, and P K Singhal***

Abstract

Afforestation is the most effective technique to reclaim the reduced ecosystem and to incorporate carbon from atmospheric CO_2 into biomass. Selection of ideal species for revegetation is very important step for restoration of reduced ecosystem. Arbuscular mycorrhizal fungi (AMF) are able to thrive well on poor physical substrates by extending the hyphal network to the establishing plants.

The present paper deals with the microbial development and restoration of degraded coal mine spoil through establishment of dominant tree species at Northern Coalfield Limited, Singrauli. The results indicated that the microbial biomass gradually increased with the age of plantations in different dominant species of the study area. It was found that older plantations had huge population of arbuscular mycorrhizal (AM) spores in different species. Similarly, colonization of AM spores in the roots was noticed. In terms of AM status and root colonization with AM fungi, the *Dalbergia sissoo* proved most promising species, followed by *Pongamia pinnata* and other species. These species were good for nitrogen fixation. With increasing the age of species, the microbial biomass also increased in different species. The role of AM fungi was found to recover the nutrients status of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained significant positive correlation with density of AM fungi and microbial biomass in different dominant species.

Keywords: Arbuscular mycorrhizal fungi, reduced ecosystem, restoration, nutritional characteristics, mycorrhizal succession.

Introduction

With the planned increase in coal production, more and more land is being brought under mining operation. The most serious impact of mining is land degradation, and habitat destruction of the ecosystem. These lands are chemically, physically and biologically unstable and deficient. Managed forests have the potential to conserve and sequester carbon, and thus, mitigate CO_2 emission by an amount equivalent to 11–15% of the fossil fuel emission. According to preliminary estimates by IPCC, about 60 to 90giga tonne (Gt) of carbon emission can be reduced or sequestered by slowing deforestation, establishing plantation forests, and forest regeneration between the period 1995 and 2050 (Houghton 1995; Singh and Lal, 2000). Globally, forests contain 54% of the worldwide carbon pool (2200 Gt) of terrestrial ecosystems (FAO 2001). These sequester 01 to 03Gtcarbon annually through the combined effect of reforestation, regeneration, and enhanced growth of existing forests, offset the global CO_2 emissions from deforestation (Pandey 2002). One tonne of carbon can

* State Forest Research Institute, Jabalpur, Madhya Pradesh 482008

** Rani Durgawati University, Jabalpur, Madhya Pradesh 482001

E-mail: pribohre@gmail.com, chaubey.dr@gmail.com, jamaluddin_125@hotmail.com , pksinghalrdvv@gmail.com

be sequestered by 2.2 tonnes of wood as far as Indian forests are concerned (Chaturvedi 1994).

Afforestation is the most effective technique to reclaim mined overburdens and to incorporate carbon from atmospheric CO_2 into biomass. Selection of ideal species for revegetation of mined out areas is a very important step in restoration of a reduced ecosystem.

Arbuscular mycorrhizal fungi (AMF) can thrive well on poor physical substrates by extending the hyphal network to establish an association with the plants. These fungi provide nutrients and make them available to plants in an utilizable form. Mycorrhizal fungi are among the major beneficial components of the soil microbial community which contribute to plant growth and survival by reducing stresses through symbiosis (Sylvia and Williams, 1992). These symbionts share a special nutritional relationship. The high phosphorous requirement of the nitrogen fixing root nodules of higher plants is fulfilled by the fungi; in turn the plants provide nitrogen to the chitin walled vesicular arbuscular mycorrhiza (VAM). Both the species have high carbon requirements. VAM mycelia can extend to a long distance and can link the rhizosphere and mycorrhizosphere of different plant species. It can make a common pool of the available nutrients of other plant species (Norris *et al.*1994). This way, the nutrients released into the overlapping mycorrhizosphere by plant root exudation or by root and nodule decay become available for non-N_2 fixing plants (Simard *et al.* 1997), which can play an important role in facilitating the survival and growth of other plant species. VAM helps in eco-restoration by enabling host plant to establish itself in degraded soil and by improving soil quality and health (phytoremediation). Further, AM fungi are capable of increasing soil nitrogen, organic matter-and restoring native micro flora (Sandra Brawn 2002; Chaubey *et al.* 2012). The production of soil protein glomalin in the rhizosphere increases the soil and nutrient binding capacity of the plants (Tran Van Con 2001; Vo Dai Hai 2009). The present paper deals with the microbial development and restoration of degraded coal mine through establishment of dominant tree species at Northern Coalfield Limited, Singrauli.

Materials and methods

Singrauli (24° 46' 60"- 24° 78' 33"N, 82° 49' 59"- 82° 83' 30"E, 275 -500m AMSL) was granted district status on May 24, 2008, with its headquarters at Waidhan. It has a tropical climate with a mean maximum and minimum temperature of 48° and 21°C, respectively. The area receives an average rainfall of 1000 mm. 95% precipitation occurs in rainy season. Vegetation during the pre-mining period was very dense. The land was covered with northern tropical dry sal forests (5 B/C) and northern tropical dry mixed deciduous forests (5 B/C 2). Due to mining, large forest areas were clear felled. The present study covered artificial plantations raised in the mined out Northern Coalfield Limited (NCL) area.

To analyze the influence of tree cover on soil and biological properties, soil samples from the rhizosphere of different species were collected from the surface soil upto a depth of 30cm. Five surface soil samples from different aged plantations of each species were collected and mixed thoroughly to get a composite sample. They were then divided into three replicates for analysis of physico-chemical and biological properties. Soil organic carbon was determined by standard methods (Black 1956). The nutritional properties (N, P, K) of soil were analyzed by standard soil testing methods (Jackson1976; Piper 1950).

The chloroform fumigation extraction method as proposed by Carter (1991) was used to estimate microbial biomass. The microbial biomass was expressed on an oven dry (105° C for 24 hours) soil basis.

As part of the study of biological properties, spore count of VAM and VAM root infection were assessed for rhizospheric soil of different dominant tree species planted on different overburdened mine sites.

100gm of rhizospheric soil was processed to extract live spores using the method suggested by Gerdemann and Nicholson (1963). The root samples were properly washed under running tap water and cleared by boiling in 10% aqueous KOH solution for 24 hours. The roots were again washed and stained in trypan blue, followed by a washing in distilled water. They were then mounted in lectophenol mountant (Philip and Hayman 1970). Usual microscopic techniques were followed to examine the infection in the root of different plant species. Percentage infection was calculated as follows:

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

Microbial biomass and other biological properties were studied and correlated with the nutritional characteristics.

Results

The species wise results obtained for the above parameters are discussed below:

1. *Tectona grandis* Linn. f. (Teak)

The results (Table 1) indicated that the density of AM fungi, percent root colonization and microbial biomass were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.27 (2 years old plantation) to 2.56 spores g^{-1} (18 years old plantation). The percent root colonization ranged from 53.2 (2 years old plantation) to 72.3 (18 years old plantation). The microbial biomass improved with the age of the plantation from 40.2 (2 years old plantation) to 51.5 mg kg^{-1} (18 years old plantation).

In *Tectona grandis,* the most significant correlation was found between density of AM fungi and microbial biomass (R^2=0.989, r=0.994), followed by organic carbon and available phosphorus (R^2=0.966, r=0.982), available nitrogen and microbial biomass (R^2=0.942, r=0.970), available phosphorus and microbial biomass (R^2=0.725, r=0.851), and organic carbon and microbial biomass (R^2=0.633, r=0.796). However, the variation between available nitrogen and available phosphorus, and organic carbon and available nitrogen was relatively low compared to the above mentioned characters. The variation in density of AM fungi and microbial biomass (99%), available nitrogen and microbial biomass (97%), and available phosphorus and microbial biomass (85%) could be good predictors that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen, and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

2. *Dalbergia sissoo* Roxb.

The results (Table 2) indicated that the density of AM fungi, percent root colonization, and microbial biomass were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.8 (2 years old plantation) to 3.6 spores g^{-1} (18 years old plantation). The percent root colonization ranged from 59.3 (2 years old plantation) to 71.7 (18 years old plantation). The microbial biomass improved with the age of the plantation from 32.5 (2 years old plantation) to 66.6 mg kg^{-1} (18 years old plantation).

In *Dalbergia sissoo,* the most significant correlation was found between the density of AM fungi and microbial biomass (R^2=0.839, r= 0.916), followed by organic carbon and available phosphorus (R^2=0.826, r=0.909), available phosphorus and microbial biomass (R^2=0.825, r=908), available nitrogen and microbial biomass (R^2=0.804, r=0.897) and available nitrogen and available phosphorus (R^2=0.779, r=0.883). However, the variation between organic carbon and available nitrogen (R^2=0.568, r= 0.753) and organic carbon and microbial biomass (R^2=0.514, r= 0.717) was relatively low compared to the above mentioned characters. The variation in density of AM fungi and microbial biomass (92%), and available nitrogen and microbial biomass (90%), and available phosphorus and microbial biomass (91%) could be good predictors that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

3. *Azadirachta indica* A. Juss.

The results (Table 3) indicated that the density of AM fungi, percent root colonization and microbial biomass were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.18 (2 years old plantation) to 2.28 spores g^{-1} (18 years old plantation). The percent root colonization ranged from 30.5 (2 years old plantation) to 44.8 (18 years old plantation). The microbial biomass improved with the age of the plantation from 21.2 (2 years old plantation) to 52.7 mg kg^{-1} (18 years old plantation).

In *Azadirachta indica,* the most significant correlation was found between the density of AM fungi and microbial biomass (R^2=0.985, r=0.992) followed by organic carbon and microbial biomass (R^2=954, r= 0.977), available phosphorus and microbial biomass (R^2=0.912, r=0.955), available nitrogen and available phosphorus (R^2=0.912, r=0.955), and organic carbon and available phosphorus (R^2=0.892, r=0.944). However, the variation between organic carbon and available nitrogen (R^2=0.786, r= 0.886), and available nitrogen and microbial biomass (R^2=0.755, r= 0.869) was relatively low compared to the above mentioned characters. The variation in density of AM fungi and microbial biomass (i.e. 99%), available phosphorus and microbial biomass (95%), and available nitrogen and microbial biomass (87%) could be good predictors that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

4. *Cassia siamea* Lamk

The results (Table 4) indicated that the density of AM fungi, percent root colonization and microbial biomass

were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.50 (2 years old plantation) to 2.40 spores g^{-1} (19 years old plantation). The percent root colonization ranged from 50.2 (2 years old plantation) to 69.7 (19 years old plantation). The microbial biomass improved with the age of the plantation from 35.5 (2 years old plantation) to 50.3 mg kg^{-1} (19 years old plantation).

In *Cassia siamea,* the most significant correlation was found between organic carbon and available nitrogen (R^2=0.987, r= 0.993) followed by available nitrogen and available phosphorus (R^2=0.983, r= 0.991), organic carbon and available phosphorus (R^2=0.956, r= 0.978), density of AM fungi and microbial biomass (R^2=0.923, r= 0.961), available phosphorus and microbial biomass (R^2=0.825, r= 0.908), available nitrogen and microbial biomass (R^2=0.809, r= 0.899), and organic carbon and microbial biomass (R^2=787, r= 0.887). The variation in density of AM fungi and microbial biomass (96%), available phosphorus and microbial biomass (91%), and available nitrogen and microbial biomass (90%) could be good predictors that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

5. *Pongamia pinnata* (Linn) Pierre

The results (Table 5) indicated that the density of AM fungi, percent root colonization and microbial biomass were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.2 (2 years old plantation) to 3.1 spores g^{-1} (18 years old plantation). The percent root colonization ranged from 28.3 (2 years old plantation) to 48.4 (18 years old plantation). The microbial biomass improved with the age of the plantation from 24.2 (2 years old plantation) to 54.7 mg kg^{-1} (18 years old plantation).

In *Pongamia pinnata,* the most significant correlation was found between density of AM fungi and microbial biomass (R^2=0.956, r= 0.978) followed by available phosphorus and microbial biomass (R^2=0.922, r=0.960), available nitrogen and available phosphorus (R^2=0.920, r=0.959), available nitrogen and microbial biomass (R^2=0.913, r=0.956), organic carbon and available phosphorus (R^2=0.911, r= 0.954), organic carbon and available nitrogen (R^2=0.871, r= 0.933), and organic carbon and microbial biomass (R^2=793, r= 0.890). The variation in density of AM fungi and microbial biomass (98%), available phosphorus and microbial biomass (96%), and available nitrogen and microbial biomass (95%) could be good predictors that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

6. *Gmelina arborea* Roxb.

The results (Table 6) indicated that the density of AM fungi, percent root colonization and microbial biomass were increasing with the age of plantations. The density of AM spores were recorded in the rhizosphere of all the plantations ranging from 1.6 (6 years old plantation) to 2.7 spores g^{-1} (10 years old plantation). The percent root colonization ranged from 45.5 (6 years old plantation) to 53.7 (10 years old plantation). The microbial biomass improved with the age of the plantation from 31.7 (6 years old plantation) to 42.6 mg kg^{-1} (10 years old plantation).

In *Gmelina arborea,* the most significant correlation was found between available nitrogen and available phosphorus (R^2=0.998, r= 0.999) followed by available phosphorus and microbial biomass (R^2=0.997, r=0.998), available nitrogen and microbial biomass (R^2=0.991, r=0.995), density of AM fungi and microbial biomass (R^2=0.967, r=0.983), organic carbon and microbial biomass (R^2=929, r=0.964), organic carbon and available phosphorus (R^2=0.903, r= 0.950), and organic carbon and available nitrogen (R^2=0.875, r= 0.935). The variation in available phosphorus and microbial biomass (100%), available nitrogen and microbial biomass (99%), and density of AM fungi and microbial biomass (98%) could be good indicator that this species will enhance the restoration of mine spoil. The nutritional characteristics like organic carbon, available nitrogen and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species (Table 7).

Discussion

Soil microorganisms play a significant role in soil fertility and ecosystem functioning. Plant species are supported by the soil alone they cannot thrive without microbes and their functions (Kennedy and Smith 1995). Soil microbial biomass measurements were useful in determining the degree of disturbance as well as the subsequent recovery of degraded ecosystems. Microbial biomass controls the major processes involved in nutrient transformation, cycling, organic matter, management and macro-aggregation for

favourable water and aeration characteristics (Singh and Goel 2009; Hargreaves *et al.* 2003). There was gradual increase in microbial biomass from younger to older plantations in different species. It ranged from 40.2 (2 years old plantation) to 51.5 mg kg^{-1} (18 years old plantation) in *T.grandis;* from 32.5 (2 years old plantation) to 66.6 mg kg^{-1} (18 years old plantation) in *D.sissoo;* from 21.2 (2 years old plantation) to 52.7 mg kg^{-1} (18 years old plantation) in *A.indica;* from 35.5 (2 years old plantation) to 50.3 mg kg^{-1} (19 years old plantation) in *C.siamea;* from 24.2 (2 years old plantation) to 54.7 mg kg^{-1} (18 years old plantation) in *P.pinnata;* and from 31.7 (6 years old plantation) to 42.6 mg kg^{-1} (10 years old plantation) in *G.arborea.* It was primarily due to increase in microbial population in older plantations as the availability of organic matter and humus had increased over a period of time. It was found that older plantations had a huge population of arbuscular mycorrhizal (AM) spores in different species as observed in the results as well. In younger plantations, the AM spores gradually decreased due to lesser availability of carbon and other nutrients. Similarly, colonization of AM spores in the roots was noticed. In older plantations, all five genera of AM fungi including *Acaulospora* sp., *Glomus* sp., *Gigaspora* sp., *Scutellospora* sp. and *Sclerocystis* sp. were recorded. Out of these, *Acaulospora* and *Glomus* had a high frequency. The spore population was more in *Acaulospora* and *Glomus* compared to *Gigaspora* and *Scutellospora* species. *Sclerocystis* was recorded in a few samples, especially in older plantations.
In terms of AM status and root colonization with AM fungi, *Dalbergia sissoo* proved the most promising species, followed by *Pongamia pinnata* and the others. These species were good for nitrogen fixation. With the increasing the age of the species, the microbial biomass also increased in different species. The findings were comparable with the observations recorded by Daft and Nicoloson (1974), Gupta and Shukla (1991), Jamaluddin and Chandra (2009), and Chaubey *et al.* (2012) in different studies regarding VAM status and plant succession. The VAM fungi were found to recover the nutrient status of coalmine spoil soil.

The nutritional characteristics like organic carbon, available nitrogen, and available phosphorus maintained a significant positive correlation with the density of AM fungi and microbial biomass in different dominant species. The results indicated that organic carbon, available nitrogen and available phosphorus were good indices of microbial biomass and density of AM fungi. However, the best positive correlation between microbial biomass and nutritional characteristics was found with available phosphorus followed by available nitrogen, and available carbon in different dominant species. This was attributed to the fact that to obtain more phosphorus, plants established a symbiotic relationship with the AM fungi, and thus, increased surface area of root (Curl and Truelove 1986; Uren and Reisenaur 1988; Nguyen Khac Hien 2003). The results were also in agreement with the observation of Banerjee *et al.* (2000), who reported a significant positive correlation between the number of organisms and organic carbon in coal mine spoil of Gevra Colliery. They reported that the number of organisms varied from species to species, and the differences were significant. Soil microbial biomass and AM fungi were recognized as the driving force behind nutrient transformation in soil and thus, have a major role in soil fertility and ecosystem functioning. Soil microbial biomass was also useful in determining the degree of recovery of the degraded ecosystem and nutritional budget. Microbial activity improved gradually during restoration of mine spoils (Stroo and Jeneks 1982).

Table 1. Physico-chemical and biological properties of soil in plantation forests of *Tectona grandis* Linn. f. (Teak) (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	2 years	8 years	9 years	10 years	18 years
1.	Density of AM fungi (No. of spores g^{-1})	1.27 ±0.20	2.00 ±0.26	2.10 ±0.36	2.36 ±0.11	2.56 ±0.12
2.	Percent root colonization with AM fungi	53.2 ± 3.72	57.3 ±4.58	60.4 ±5.44	62.5 ±3.75	72.3 ±5.06
3.	Microbial biomass (mg kg^{-1})	40.2 ± 2.41	46.1 ±3.23	47.4 ±3.79	48.7 ±3.90	51.5 ±4.64
4.	Organic carbon (%)	0.82 ±0.03	0.9 ±0.05	0.93 ±0.06	1.84 ±0.16	2.2 ±0.09
5.	Available nitrogen (kg ha^{-1})	180 ±7.54	345 ±10.00	355 ±10.00	398 ±11.13	410 ±18.02
6.	Available phosphorus(kg ha^{-1})	8.6 ±0.70	8.82 ±0.45	8.85 ±0.40	9.5 ±0.47	10.12 ±0.96

Table 2. Physico-chemical and biological properties of soil in plantation forests of *Dalbergia sissoo*Roxb. (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	2 years	4 years	5 years	6 years	7 years	8 years	9 years	10 years	11 years	13 years	14 years	15 years	16 years	18 years
1.	Density of AM fungi (No. of spores g^{-1})	1.8 ± 0.11	2.1 ± 0.15	2.2 ± 0.11	2.25 ± 0.14	2.31 ± 0.18	2.65 ± 0.19	2.80 ± 0.14	2.91 ± 0.20	2.95 ± 0.18	2.97 ± 0.21	2.99 ± 0.24	3.1 ± 0.28	3.3 ± 0.16	3.6 ± 0.25
2.	Percent root colonization with AM fungi	59.3 ± 4.15	60.2 ± 4.82	61.3 ± 4.90	63.2 ± 4.42	64.3 ± 5.14	64.5 ± 5.81	64.6 ± 5.17	65.4 ± 4.58	65.4 ± 3.92	65.9 ± 3.30	70.1 ± 4.21	70.3 ± 4.92	70.5 ± 4.94	71.7 ± 5.74
3.	Microbial biomass (mg kg^{-1})	32.5 ± 2.28	34.3 ± 2.40	36.4 ± 2.91	38.6 ± 2.70	39.7 ± 2.78	40.2 ± 3.22	41.8 ± 2.93	42.5 ± 3.40	43.6 ± 3.05	44.5 ± 3.56	47.2 ± 3.78	51.3 ± 3.59	54.5 ± 3.27	66.6 ± 5.33
4.	Organic carbon (%)	0.36 ± 0.04	0.48 ± 0.06	0.5 ± 0.07	1 ± 0.13	1.44 ± 0.19	1.44 ± 0.19	1.46 ± 0.19	1.49 ± 0.21	1.52 ± 0.22	1.56 ± 0.23	1.6 ± 0.23	1.64 ± 0.23	1.64 ± 0.26	1.65 ± 0.23
5.	Available nitrogen (kg ha^{-1})	187 ± 15.23	215 ± 20.41	267 ± 18.32	225 ± 20.55	278 ± 29.57	380 ± 33.15	286 ± 30.48	288 ± 32.80	290 ± 33.10	328 ± 34.25	362 ± 35.11	363 ± 37.12	367 ± 38.68	482 ± 40.40
6.	Available phosphorus (kg ha^{-1})	6.67 ± 0.48	7.6 ± 0.54	10.9 ± 0.78	11.9 ± 0.85	12.9 ± 0.93	13.4 ± 0.96	14.4 ± 1.04	14.5 ± 1.08	14.8 ± 1.15	15.8 ± 1.17	16.3 ± 1.17	17.6 ± 1.22	17.9 ± 1.30	20 ± 1.45

Table 3. Physico-chemical and biological properties of soil in plantation forests of *Azadirachta indica* A. Juss. (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	2 years	5 years	6 years	7 years	8 years	10 years	14 years	18 years
1.	Density of AM fungi (No. of spores g^{-1})	1.18 ± 0.07	1.23 ± 0.07	1.45 ± 0.10	1.48 ± 0.12	1.50 ± 0.09	1.70 ± 0.09	2.00 ± 0.12	2.28 ±0.16
2.	Percent root colonization with AM fungi	30.5 ± 2.14	32.2 ± 2.25	35.4 ± 2.83	36.6 ± 3.29	37.5 ± 3.00	40.6 ± 3.25	41.5 ± 2.91	44.8 ± 2.69
3.	Microbial biomass (mg kg^{-1})	21.2 ± 1.48	25.1 ± 2.01	29.3 ± 2.64	30.7 ± 2.46	32.6 ± 2.28	36.2 ± 3.26	42.5 ± 3.40	52.7 ± 4.22
4.	Organic carbon (%)	0.73 ± 0.06	1.35 ± 0.09	1.69 ± 0.10	1.73 ± 0.14	1.79 ± 0.11	1.95 ± 0.14	2.25 ± 0.16	3.05 ± 0.27
5.	Available nitrogen (kg ha^{-1})	164 ± 11.48	225 ± 18.00	267 ± 26.70	329 ± 23.03	358 ± 28.68	365 ± 29.20	390 ± 35.10	400 ± 38.50
6.	Available phosphorus (kg ha^{-1})	8.33 ± 0.75	9.55 ± 0.96	9.9 ± 0.89	10.7 ± 0.64	11.12 ± 1.11	12.3 ± 1.11	12.6 ± 1.26	13.5 ± 1.49

Table 4. Physico-chemical and biological properties of soil in plantation forests of *Cassia siamea* Lamk (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	2 years	6 years	11 years	14 years	15 years	19 years
1.	Density of AM fungi (No. of spores g^{-1})	1.50 ± 0.09	1.70 ± 0.12	1.90 ± 0.13	2.00 ± 0.16	2.10 ± 0.15	2.40 ± 0.22
2.	Percent root colonization with AM fungi	50.2 ± 3.51	54.5 ± 4.36	58.4 ± 4.67	61.6 ± 4.31	62.3 ± 5.61	69.7 ± 5.58
3.	Microbial biomass (mg kg^{-1})	35.5 ± 2.49	41.6 ± 2.91	44.5 ± 3.56	46.4 ± 4.18	48.7 ± 4.38	50.3 ± 5.03
4.	Organic carbon (%)	0.23 ± 0.02	0.25 ± 0.03	0.28 ± 0.02	0.39 ± 0.03	0.47 ± 0.03	0.56 ± 0.04
5.	Available nitrogen (kg ha^{-1})	137 ± 6.02	142 ± 7.10	149 ± 11.92	161 ± 11.27	181 ± 14.48	193 ± 15.44
6.	Available phosphorus(kg ha^{-1})	17.5 ± 1.58	18 ± 1.26	19.2 ± 1.54	19.57 ± 1.57	21.52 ± 1.72	22.95 ± 1.84

Table 5. Physico-chemical and biological properties of soil in plantation forests of *Pongamia pinnata* (Linn) Pierre (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	2 years	4 years	5 years	6 years	8 years	9 years	10 years	13 years	14 years	15 years	18 years
1.	Density of AM fungi (No. of spores g^{-1})	1.20 ± 0.06	1.35 ± 0.07	1.40 ± 0.08	1.48 ± 0.09	1.80 ± 0.12	1.90 ± 0.13	1.97 ± 0.13	2.10 ± 0.14	2.80 ± 0.19	2.90 ± 0.21	3.10 ± 0.23
2.	Percent root colonization with AM fungi	28.3 ± 1.53	31.4 ± 1.76	32.2 ± 2.06	35.7 ± 2.46	37.8 ± 2.72	40.3 ± 2.90	41.9 ± 3.27	43.5 ± 3.44	46.3 ± 3.80	46.5 ± 3.86	48.4 ± 4.21
3.	Microbial biomass (mg kg^{-1})	24.2 ± 1.74	26.3 ± 2.05	27.6 ± 2.26	29.4 ± 2.44	32.7 ± 2.91	35.8 ± 3.29	37.2 ± 3.46	40.4 ± 3.80	43.9 ± 4.48	46.6 ± 4.80	54.7 ± 6.02
4.	Organic carbon (%)	0.42 ± 0.03	0.89 ± 0.07	0.96 ± 0.08	0.99 ± 0.09	1.15 ± 0.09	1.16 ± 0.10	1.22 ± 0.12	1.26 ± 0.13	1.29 ± 0.13	1.32 ± 0.13	1.57 ± 0.14
5.	Available nitrogen (kg ha^{-1})	136 ± 10.88	164 ± 13.12	184 ± 16.54	258 ± 23.22	268 ± 18.76	298 ± 20.86	305 ± 21.35	326 ± 26.08	330 ± 29.70	334 ± 30.06	446 ± 31.22
6.	Available phosphorus (kg ha^{-1})	8.53 ± 0.68	9.2 ± 0.83	10.2 ± 0.71	10.3 ± 0.83	10.8 ± 0.97	11 ± 0.99	11.2 ± 1.01	11.6 ± 0.81	12.1 ± 0.85	12.4 ± 1.12	13 ± 1.04

Table 6. Physico-chemical and biological properties of soil in plantation forests of *Gmelina arborea* Roxb. (*n*=3 in each plantation; values are mean ± standard deviation)

S.No.	Parameters	6 years (2003)	9 years (2000)	10 years (1999)
1.	Density of AM fungi (No. of spores g^{-1})	1.6 ± 0.12	2.2 ± 0.17	2.7 ± 0.22
2.	Percent root colonization with AM fungi	45.5± 3.78	50.6 ± 4.50	53.7 ± 4.94
3.	Microbial biomass (mg kg^{-1})	31.7± 2.95	39.4 ± 3.70	42.6 ± 4.35
4.	Organic carbon (%)	0.3 ± 0.02	0.68 ± 0.05	1.11 ± 0.11
5.	Available nitrogen (kg ha^{-1})	150 ± 9.50	245 ± 20.83	270 ± 22.95
6.	Available phosphorus(kg ha^{-1})	9.4 ± 0.70	9.85 ± 0.79	10 ± 0.80

Table 7. Correlation between nutritional and microbial characteristics of dominant species

			Organic carbon (%)	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Density of AM fungi (No. of spores g^{-1})	Microbial biomass (mg kg^{-1})
Tectona grandis	Organic carbon (%)	Pearson correlation	1	0.701**	0.783**	0.781**	0.779**
		Sig. (2-tailed)		0.004	0.001	0.001	0.001
		N	15	15	15	15	15
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.701**	1	0.614*	0.958**	0.948**
		Sig. (2-tailed)	0.004		0.015	0.000	0.000
		N	15	15	15	15	15
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.783**	0.614*	1	0.774**	0.766**
		Sig. (2-tailed)	0.001	0.015		0.001	0.001
		N	15	15	15	15	15
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.781**	0.958**	0.774**	1	0.982**
		Sig. (2-tailed)	0.001	0.000	0.001		0.000
		N	15	15	15	15	15
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.779**	0.948**	0.766**	0.982**	1
		Sig. (2-tailed)	0.001	0.000	0.001	0.000	
		N	15	15	15	15	15

			Organic carbon (%)	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Density of AM fungi (No. of spores g^{-1})	Microbial biomass (mg kg^{-1})
Dalbergia sissoo	Organic carbon (%)	Pearson correlation	1	0.776**	0.912**	0.857**	0.733**
		Sig. (2-tailed)		0.000	0.000	0.000	0.000
		N	42	42	42	42	42
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.776**	1	0.882**	0.880**	0.894**
		Sig. (2-tailed)	0.000		0.000	0.000	0.000
		N	42	42	42	42	42
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.912**	0.882**	1	0.910**	0.913**
		Sig. (2-tailed)	0.000	0.000		0.000	0.000
		N	42	42	42	42	42
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.857**	0.880**	0.910**	1	0.870**
		Sig. (2-tailed)	0.000	0.000	0.000		0.000
		N	42	42	42	42	42
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.733**	0.894**	0.913**	0.870**	1
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	
		N	42	42	42	42	42
Azadirachta indica	Organic carbon (%)	Pearson correlation	1	0.897**	0.947**	0.944**	0.966**
		Sig. (2-tailed)		0.000	0.000	0.000	0.000
		N	24	24	24	24	24
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.897**	1	0.958**	0.865**	0.859**
		Sig. (2-tailed)	.000		0.000	0.000	0.000
		N	24	24	24	24	24
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.947**	0.958**	1	0.947**	0.935**
		Sig. (2-tailed)	0.000	0.000		0.000	0.000
		N	24	24	24	24	24
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.944**	0.865**	0.947**	1	0.938**
		Sig. (2-tailed)	0.000	0.000	0.000		0.000
		N	24	24	24	24	24
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.966**	0.859**	0.935**	0.938**	1
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	
		N	24	24	24	24	24
Cassia siamea	Organic carbon (%)	Pearson correlation	1	0.952**	0.954**	0.901**	0.896**
		Sig. (2-tailed)		0.000	0.000	0.000	0.000
		N	18	18	18	18	18
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.952**	1	0.984**	0.960**	0.892**
		Sig. (2-tailed)	0.000		0.000	0.000	0.000
		N	18	18	18	18	18
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.954**	0.984**	1	0.971**	0.915**
		Sig. (2-tailed)	0.000	0.000		0.000	0.000
		N	18	18	18	18	18
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.901**	0.960**	0.971**	1	0.931**
		Sig. (2-tailed)	0.000	0.000	0.000		0.000
		N	18	18	18	18	18
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.896**	0.892**	0.915**	0.931**	1
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	
		N	18	18	18	18	18

			Organic carbon (%)	Available nitrogen (kg ha^{-1})	Available phosphorus (kg ha^{-1})	Density of AM fungi (No. of spores g^{-1})	Microbial biomass (mg kg^{-1})
Pongamia pinnata	Organic carbon (%)	Pearson correlation	1	0.934**	0.960**	0.870**	0.874**
		Sig. (2-tailed)		0.000	0.000	0.000	0.000
		N	33	33	33	33	33
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.934**	1	0.948**	0.908**	0.950**
		Sig. (2-tailed)	0.000		0.000	0.000	0.000
		N	33	33	33	33	33
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.960**	0.948**	1	0.946**	0.924**
		Sig. (2-tailed)	0.000	0.000		0.000	0.000
		N	33	33	33	33	33
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.870**	0.908**	0.946**	1	0.962**
		Sig. (2-tailed)	0.000	0.000	0.000		0.000
		N	33	33	33	33	33
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.874**	0.950**	0.924**	0.962**	1
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	
		N	33	33	33	33	33
Gmelina arborea	Organic carbon (%)	Pearson correlation	1	0.999**	0.998**	0.134	0.294
		Sig. (2-tailed)		0.000	0.000	0.731	0.443
		N	9	9	9	9	9
	Available nitrogen (kg ha^{-1})	Pearson correlation	0.999**	1	0.999**	0.108	0.270
		Sig. (2-tailed)	0.000		0.000	0.782	0.483
		N	9	9	9	9	9
	Available phosphorus (kg ha^{-1})	Pearson correlation	0.998**	0.999**	1	0.093	0.258
		Sig. (2-tailed)	0.000	0.000		0.812	0.502
		N	9	9	9	9	9
	Density of AM fungi (No. of spores g^{-1})	Pearson correlation	0.134	0.108	0.093	1	0.973**
		Sig. (2-tailed)	0.731	0.782	0.812		0.000
		N	9	9	9	9	9
	Microbial biomass (mg kg^{-1})	Pearson correlation	0.294	0.270	0.258	0.973**	1
		Sig. (2-tailed)	0.443	0.483	0.502	0.000	
		N	9	9	9	9	9

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
N= Sample no. in dominant species.

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Effect of salinity stress on growth and antioxidant enzyme activities in tomato plants inoculated with Glomus intraradices

Mayura Dudhane[1], Mahesh Borde[2], P K Jite[2] and M V Kulkarni[1]*

Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops in India. Arbuscular mycorrhizal (AM) fungi are soil microorganisms that establish mutual symbiosis with majority of higher plants, providing a direct physical link between soil and plant roots. To study the growth and antioxidant enzyme activities in tomato plants under salinity stress conditions, three different concentrations (100 mM, 200 mM and 300 mM) of NaCl were given to AM inoculated and non-AM inoculated tomato seedlings. In the present study, mycorrhizal tomato plants showed significant increase in shoot length, root length, fresh weight and dry weight. AM colonization significantly increased antioxidant enzyme activities like superoxide dismutase and peroxidase activity in mycorrhizal tomato plants as compared to non-AM inoculated plants under different salinity levels, thus rendering the former (mycorrhizal) tomato plants to be sturdier and productive as compared to the latter (non mycorrhizal) ones.

Keywords: Salinity, mycorrhizal fungi, antioxidant enzymes

Introduction

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, particularly in arid and semi-arid areas (Abdel–Latef 2010). In India about 8.6 mha (Pathak 2000) of land area is affected by soil salinity. Salt alters a wide array of metabolic processes, culminating in stunted growth, and reduced enzyme activities and biochemical constituents, hence limiting plant growth and productivity (Ashraf and Foolad 2007).

Antioxidant mechanisms may provide a strategy to enhance salt tolerance in plants. Plants under stress produce some defence mechanisms to protect themselves from the harmful effect of oxidative stress. Reactive oxygen species (ROS) scavenging is a common defence response against abiotic stresses (Vranova *et al.* 2002). Its detoxification mechanism is achieved by integrated system of non- enzymatic antioxidants such as ascorbate and glutathione, and enzymatic antioxidants such as CAT, GPX, GR, SOD etc. (Prochazkova *et al.* 2001; Shrivali *et al.* 2003). The primary antioxidant enzyme that converts superoxide to H_2O_2 and oxygen is SOD (Alscher *et al.* 2002).

Tomato (*Lycopersicon esculentum* L.) is considered a major vegetable crop in many parts of the world. It is mostly grown under irrigation, both in protected and open field conditions (Al-Karaki 2006). It was selected as a model crop for this study due to its commercial importance as a horticultural cash crop. Tomato is moderately sensitive to salinity. Extensive study on growth conditions need to be conducted under moderate salinity to produce vegetative growth.

The present study was undertaken in an attempt to improve the survival and growth of tomato plants in a saline soil condition using Arbuscular mycorrhizal (AM) fungi.

Methods

Plant material, AM inoculum, and experimental design

Pure inoculum of mycorrhizal fungus *Glomus intraradices* was obtained from Dr D J Bagyaraj, CNRBD, Bangalore. The spores were multiplied on bajara for two months and used as an inoculum (soil containing spores AM colonized roots and extraradical mycelium) for the treatment of *Lycopersicon esculentum* seedlings. Non-mycorrhizal plants consist of the same inoculums, but are autoclaved for 1 hour at 121° C. The seedlings were grown in soil (autoclaved for1hour at 121°C) from seeds of *Lycoperiscon esculantum* (F1 hybrid variety-Abhinav, Syngenta seeds company) (brought from Agriculture College, Pune, Maharashtra, India).

Experimental design

The experimental design consisted of eight treatments having non–AM inoculated plants and AM inoculated plants with four salinity levels (NaCl: 0, 100, 200 and 300 mM in 100 ml of half strength of Hoagland nutrient solution) per 5 kg of sterilized soil. Pots were arranged in a completely randomized block design. Nine replicates of each treatment were grown. A total of 72 pots (three plants/pot) were arranged. 21-days-old tomato seedlings were used for the salinity

1 Division of Biochemistry, Department of Chemistry, University of Pune, Pune–411007.
2 Department of Botany, University of Pune, Pune–411007.
* Email: mayuradudhane@gmail.com

experiment. NaCl was used for salinity stress. After 30 days of AM inoculation, NaCl treatment was given at every eight days interval and was continued till the last observation was taken. Observations were recorded after 60, 90, and 120 days of AM inoculation.

Morphological parameters

Shoot length, root length, fresh weight, and dry weight were measured as per the standard methods. The roots were cleared and stained using the methods adopted by Philips and Hayman (1970), and the percentage of mycorrhizal colonization was estimated by the gridline intersect method (Giovannetti and Mosse 1980).

Enzyme extraction

500mg of fresh shoot and root tissue were ground in 3ml of extraction buffer in a pre–cooled mortar and pestle. The homogenate was centrifuged at 18000g at 5°C for 10 minutes. The supernatant obtained was used for enzyme assay.

Determination of guaiacol peroxidase (GPX)

GPX specific activity was determined by monitoring guaiacol oxidation as described by Putter (1974). The reaction mixture was composed of 0.1 ml of 0.2 M guaiacol, 1ml of 0.1 M PO4 buffer (pH 6.1), 500ml of H2O2 and 20ml of enzyme extract. The final volume was made up to 3ml with distilled water. An increase in absorbance at 436 nm was determined after 2 minutes incubation. GPX activities were expressed in M Kat/gm.

Determination of superoxide dismutase (SOD)

SOD activity was determined photochemically by using an assay system consisting of methionine, riboflavin and NBT. The original assay described by Beauchamp and Fridovich (1971) was modified. The reaction mixture was composed of 1.3mM riboflavin, 13mM methionine, 63mM NBT, 0.05M sodium carbonate (pH 10.2) and 50 ml enzyme extract. Distilled water was added to bring the final volume up to 3ml. The initial rate of reaction was determined as increase of absorbance at 560 nm. SOD activity was expressed in Units. One unit (U) is defined as the amount of absorbance change per mg fresh weight per hour.

Statistical analysis

Statistical analysis was performed using one–way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT). The values are mean ± standard deviation for six treatments in each group. *P* values ≤ 0.05 were considered significant.

Result

Shoot length consequently increased with increasing NaCl concentration after 60, 90, and 120 days of AM inoculation in mycorrhizal tomato plants as compared to non–mycorrhizal tomato plants. When compared to control plants, AM inoculated tomato plants showed 69% shoot increment at zero salinity level after 60 days, 66% shoot increment at first salinity level after 90 days, and 68% shoot increment at first level of salinity after 120 days of AM inoculation. Root length increased with increasing concentration of NaCl at first (100mM) and second (200mM) salinity levels, but found to decline at high salinity levels (300mM) after 60, 90, and 120 days in both AM inoculated and non–AM inoculated tomato plants. However root length was found to be more in AM inoculated tomato plants compared to non–AM inoculated tomato plants (Table 1). Percent root increment in mycorrhizal tomato plants was 81% at zero salinity level after 60 days, and 65% at second salinity level after 90 and 120 days of AM inoculation.

In mycorrhizal tomato plants, fresh and dry weight increased significantly with increasing concentration of salinity (100mM and 200mM) after 60, 90, and 120 days of AM inoculation compared to non–mycorrhizal plants. At third salinity level (300mM), fresh and dry weight of mycorrhizal as well as non–mycorrhizal tomato plants was found to decline, but was found to be higher in mycorrhizal plants as compared to non–mycorrhizal tomato plants (Table 2).

After 60, 90, and 120 days of AM inoculation, percent root colonization in mycorrhizal tomato under non–saline conditions was 56%, 66%, and 66% respectively. In mycorrhizal tomato plants, at first salinity level, percent root colonization was 60%, 70%, and 73% after 60, 90, and 120 days of AM inoculation respectively. At second salinity level, percent root colonization was 60%, 63%, and 76% after 60, 90, and 120 days of AM inoculation respectively. At third salinity level, percent root colonization was found to be 56%, 60% and 66% after 60, 90, and 120 days of AM inoculation respectively (Fig 1).

When compared to non-mycorrhizal tomato plants, both shoot and root SOD activity in mycorrhizal tomato plants was found to increase at all salinity levels after 60, 90, and 120 days of AM inoculation (Fig. 2 and 3). Percent increment in shoot SOD activity in mycorrhizal tomato plants was 51%, 39%, and 40% at zero salinity level after 60, 90, and 120 days of AM inoculation respectively. Similarly, percent increment in root SOD activity was 67%, 67%, and 73% at zero salinity level after 60, 90, and 120 days of AM inoculation respectively.

Shoot GPX activity was found to be more in mycorrhizal tomato plants when compared to non-mycorrhizal tomato plants after 60 and 90 days of AM inoculation at all salinity levels. After 120 days of AM inoculation, shoot GPX activity was found to decline in both mycorrhizal and non-mycorrhizal tomato plants. However, activity was found to be more in mycorrhizal tomato plants (Fig. 4). Percent increment in shoot GPX activity was 58%, 85%, and 85% at first salinity level after

60, 90, and 120 days of AM inoculation respectively. Root GPX activity was consequently increased in mycorrhizal tomato plants with increasing NaCl concentration after 60, 90, and 120 days of AM inoculation as compared to non-mycorrhizal tomato plants. In mycorrhizal tomato plants, percent increment in root GPX activity was 86%, 70%, and 75% at first salinity level after 60, 90, and 120 days of AM inoculation respectively (Fig. 5).

Discussion

Salinity is one of the major limitations on crop productivity and quality throughout the world. In recent years, some studies have indicated that AMF can enhance plant growth and uptake of nutrients, decrease yield losses of tomato under saline conditions, and improve salt tolerance of tomato (Huang *et al.* 2010; Abdel Latef and Chaoxing 2011). In the present study, it was observed that high saline conditions resulted in decline in tomato growth (100mM NaCl). Increasing soil salinity had a negative effect on plant growth. Increase in height as observed in AM inoculated plants could be a result of enhanced inorganic nutrient absorption (Cooper 1984) and greater rates of photosynthesis (Allen *et al.* 1981). In the present study, it was observed that the symbiotic association between AM fungus, *Glomus intraradices,* and the tomato plant was strengthened in a saline environment resulting in increased shoot and root length and total plant biomass. Fresh and dry weight of mycorrhizal tomato plants was found to be more than non-mycorrhizal tomato plants. Similar results were observed by Al-Karaki (2000). Fresh and dry weight of tomato plants were found to decline at high salinity levels (100mM) after 60, 90, and 120 days of AM inoculation. These results are in agreement with ZhongQun *et al.* (2007), who reported that salinity reduces fresh and dry weight of tomato plants. The reduction of dry weights due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ (Hajiboland *et al.* 2010; Abdel Latef and Chaoxing 2011).

Previous studies have shown that salinity may reduce mycorrhizal colonization by inhibiting the germination of spores (Hirrel and Gerdemann 1980), growth of hyphae in soil and hyphal spreading after initial infection had occurred (McMillen *et al*. 1998), and reducing the number of arbuscules (Tian *et al*. 2004). In the present study, percent root colonization increased at low salinity levels and decreased with increasing salinity level and days of salinity treatments.

Salinity induced the production of stress proteins or antioxidant enzymes, which minimizes the damage caused by reactive oxygen species (ROS) (Porcel *et al*. 2003). Production of ROS is increased under saline conditions (Hasegawa *et al*., 2000), ROS-mediated membrane damage has been demonstrated to be a major cause of cellular toxicity by salinity in rice, tomato, and citrus plants (Mittova *et al*. 2004). In tomato, it was found that increased activities of antioxidant enzymes like SOD and GPX helps these plants to resist high salinity. Similar results were reported by Mittova *et al*. 2004. According to them, salt-tolerance is attributed to the increased activities of SOD and GPX. ZhongQun *et al*. (2007) observed growth parameters, cell membrane osmosis, and the activities of SOD, POD, APX, and CAT in roots of AM and non-AM tomato under NaCl and normal conditions. They also evaluated the effects of these enzymes in ROS scavenging on the enhanced salt tolerance by AM fungi.

Increased peroxidase activity is one of the most widespread biochemical activities in diseased and injured plant tissues. Plants with high concentration of antioxidants have been reported to have greater resistance to this oxidative damage (Jiang and Zhang 2002). Salinity results in higher activity of POD (Gossett *et al*. 1994) and SOD (Sudhakar *et al*. 2001). In tomato plants, it was found that AM colonization significantly increased SOD and GPX activity in both shoot and root.

The study showed an increased growth in tomato plants and also enhanced antioxidant activities in AM inoculated plants. In conclusion, the results confirm that arbuscular mycorrhizal fungi alleviate the detrimental effect of salinity through improved nutrient uptake. The present study revealed that exposure of mycorrhizal tomato plants to salinity resulted in significant induction of antioxidative enzyme activities such as SOD and GPX that could help the plants protect themselves from the oxidative effects of ROS. Hence, mycorrhizal inoculation helps tomato plants to survive better under saline conditions.

Acknowledgement

The authors are grateful for the financial support given by UGC under the Dr. D. S. Kothari Post Doctoral Fellowship.

Table 1: Morphological parameters of tomato after 60, 90, and 120 days of AM inoculation

Treatments	Shoot length (cm)			Root length (cm)		
	60 days	90 days	120 days	60 days	90 days	120 days
C	37.66±7.133e	39.34±1.934d	40.85±0.898h	18.33±2.054c	24.67±2.494b	25.93±0.192d
C+1S	46.66±4.109de	47.33±4.109cd	47.57±0.556g	23.66±0.942b	25.66±1.699b	26.68±0.856d
C+2S	54.66±2.867cd	58.66±2.494c	59.46±0.315f	25.33±3.091b	25.34±3.091b	25.95±0.256d
C+3S	54.33±3.681cd	64.00±3.559b	64.03±0.663e	24.00±2.494b	24.33±3.042b	24.50±1.944c
Gi	63.66±6.018bc	65.33±5.436b	66.45±1.246d	33.33±1.885a	38.00±4.966a	38.32±0.534b
Gi+1S	74.00±6.683b	79.00±3.366a	80.12±1.706c	35.33±1.247a	38.32±1.247a	38.83±0.156b
Gi+2S	77.00±2.160a	80.66±4.496a	82.95±0.296b	36.66±1.247a	42.00±3.741a	42.89±0.328a
Gi+3S	76.66±3.299a	85.33±4.921a	86.57±0.502a	36.00±3.265a	38.30±1.247a	39.96±0.727b

Note: Different letters in each individual experiment indicate significant difference between NaCl concentrations at $P \leq 0.05$.

Table 2: Morphological parameters of tomato after 60, 90, and 120 days of AM inoculation

Treatments	Fresh weight (gm)			Dry weight (gm)		
	60 days	90 days	120 days	60 days	90 days	120 days
C	6.15±0.448d	6.28±0.431d	7.06±0.182c	2.53±0.106e	2.77±0.135d	3.45±0.161c
C+1S	7.08±0.843cd	8.16±0.180c	8.54±1.439bc	2.71±0.189e	3.01±0.163cd	3.98±0.517bc
C+2S	8.67±0.899bc	9.12±0.275bc	9.54±0.186b	3.06±0.196de	3.60±0.494bc	4.32±0.445abc
C+3S	8.29±0.622bc	8.82±0.117c	9.39±0.086b	2.93±0.254e	3.57±0.190bc	4.12±0.301abc
Gi	7.89±0.670bc	10.01±0.244b	11.43±0.345a	3.54±0.414cd	3.97±0.418ab	4.45±0.656abc
Gi+1S	9.19±1.028b	10.98±0.212a	11.89±0.377a	4.17±0.235ab	4.31±0.385ab	4.85±0.526ab
Gi+2S	11.78±0.517a	11.92±1.065a	12.20±1.433a	4.27±0.286a	4.73±0.072a	5.11±0.421a
Gi+3S	11.36±1.328a	11.81±0.135a	12.00±0.234a	3.72±0.238bc	4.53±0.517a	4.84±0.439ab

Note: Different letters in each individual experiment indicate significant difference between NaCl concentrations at $P \leq 0.05$.

Fig. 1: Per cent root colonization

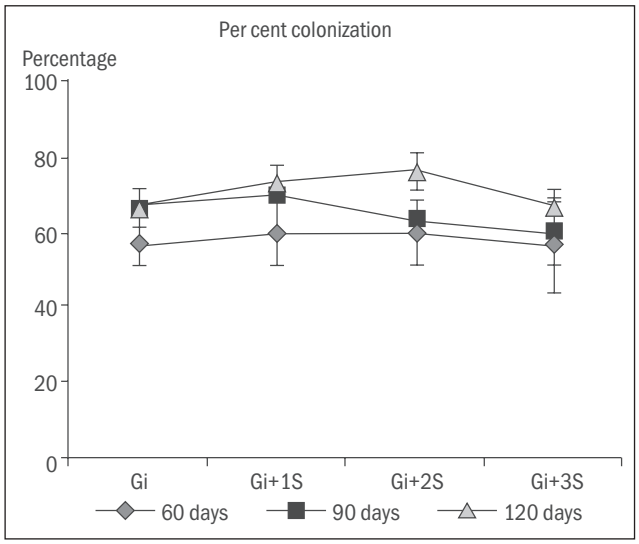


Fig. 2: Shoot SOD activity

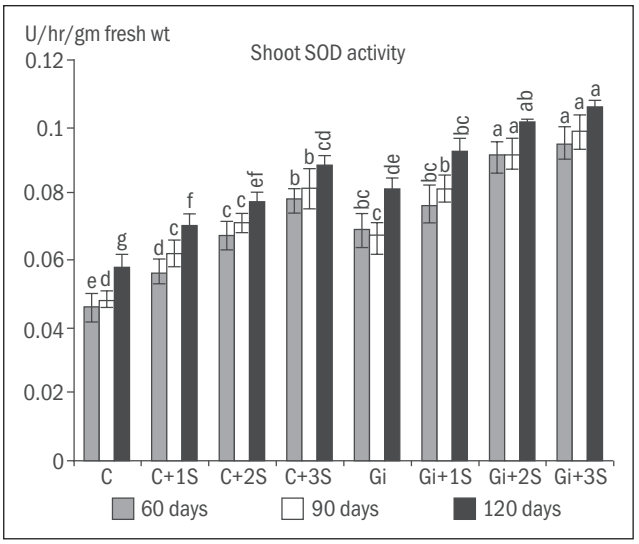


Fig. 3: Root SOD activity

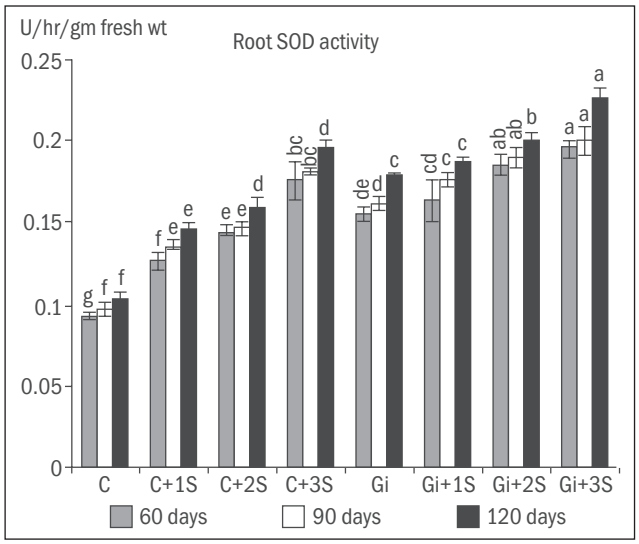


Fig. 4: Shoot GPX activity

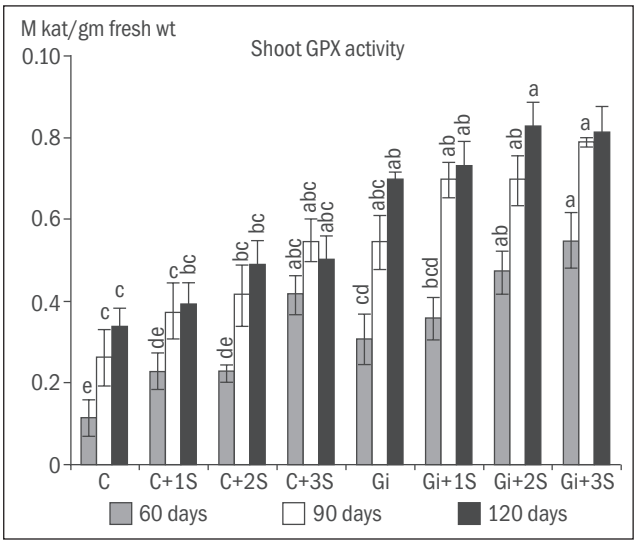
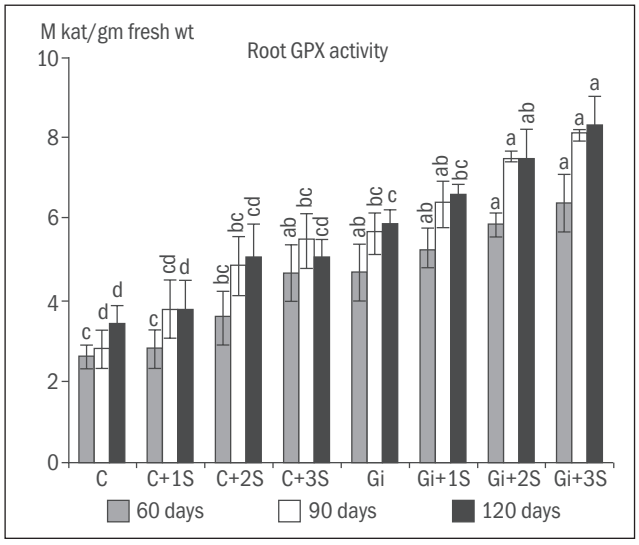


Fig. 5: Root GPX activity



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CENTRE FOR MYCORRHIZAL CULTURE COLLECTION

Showcasing Ectomycorrhizal Culture

Chaitali Bhattacharya[*] and *Alok Adholeya*[#]

It has always been our endeavour to update our readers with the new additions that has been successfully made into our ever growing Culture Collection Bank. We would like to acknowledge that we have shown a little inattention towards exhibiting one of the ensuing wealth of our collection. We intend to rectify our slip-up in this edition by showcasing the vast collection of Ectomycorrhiza (EcM) well-maintained in our culture collection bank.

An ectomycorrhizal (EcM) root is characterized by the presence of three structural components: a sheath or mantle of fungal tissue which encloses the root, a labyrinthine inward growth of hyphae between the epidermal and cortical cells called the Hartig net and an outwardly growing system of hyphal elements (the extraradical or external mycelium). The ectomycorrhizal (EcM) symbiosis involves association between plants and fungal taxa worldwide. Unlike arbuscular mycorrhizal fungi, EcM fungi reproduce sexually and produce macroscopic fruit bodies. Field observations, collections and isolations from these fruit bodies have helped us in bringing together an amazing diversity of putatively EcM forming fungi from many taxonomically genera. Appended at the end of our write up is the list of available cultures that can be provided to the researchers whenever requested.

Once a request is placed, we generate and transport two small petriplates containing actively growing mycelial biomass. These cultures should then be subcultured and maintained with utmost care under sterile conditions. The methodology for doing so is as follows:

- Prepare fresh modified Melin-Norkrans (MMN) medium (composition appended below).
- Pour the media in the petriplates inside a laminar flow and leave them for a day under sterile condition.
- Subculture the mother culture plates received from TERI under sterile condition (laminar hood) into the new media plates.
- Sub-culturing can be done by cutting the mycelial discs from the corners/edges of the mother culture plate. The cutting should be such that both fresh mycelial mat along with media is removed. A cork borer can be used for the same.
- Place the culture disc into the fresh plate such that it touches the surface of the new media. Place two to three such discs into the new plates.
- Seal the plate with parafilm and place them in the BOD at 25-26 °C under dark condition.
- Keep checking every week till 15 to 20 days (depending on the growth of the culture) for re sub-culturing.

Modified Melin-Norkrans (MMN) Medium

- Final volume: 1L MMN
- Use a graduated cylinder and measure 900 mL of distilled water (D.W.) into a 1L beaker.
- Put the beaker on a stir plate and while stirring add the following chemicals (use a pipet):

Components	Stock	Working stock
$CaCl_2 \cdot 2H_2O$ stock	1.5gm/150ml	5ml/L
NaCl stock	0.75gm/150ml	0.75gm/150ml
$MgSO_4 \cdot 7H_2O$ stock	4.50gm/150ml	5ml/L
$(NH_4)_2HPO_4$ stock	7.5gm/150ml	5ml/L
KH_2PO_4 stock	15.0gm/150ml	5ml/L
$FeCl_3 \cdot 6H_2O$	1.0gm/100ml	1.2ml/L Stored in refrigerator
Thiamine HCl	10.0mg/100ml	1.0ml/L Stored in refrigerator
Glucose (D-(+)-glucose)		10.0g/L
Malt extract		3.0g/L

[*] Research Associate, Biotechnology and Bioresources, Centre for Mycorrhizal Research, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi-110 003, India

[#] Director, Biotechnology and Bioresources, Centre for Mycorrhizal Research, The Energy and Resources Institute, Darbari Seth Block, IHC Complex, Lodhi Road, New Delhi-110 003, India

- Make the volume to one litre using D.W.
- Set the pH to 5.6.
- Add 8.0 gm/L of agar to the media.
- Autoclave the media at 121°C for 25 min.
- Routinely subculture it within 15 to 20 days.

S.No.	Bank Code	Culture Name
1.	*EM- 1001*	*Alpova diplophloeous*
2.	*EM- 1129*	*Alpova olivaceotinctus*
3.	*EM- 1145*	*Amanita murina*
4.	*EM- 1100*	*Amanita muscaria var. farmosa*
5.	*EM- 1084*	*Amanita muscaria*
6.	*EM- 1069*	*Amanita muscaria*
7.	*EM- 1146*	*Amanita muscaria*
8.	*EM- 1060*	*Amanita muscaria*
9.	*EM- 1148*	*Boletus cavipes*
10.	EM- 1277	Boletus cavipes
11.	*EM- 1072*	*Boletus edulis*
12.	*EM- 1151*	*Cenococcum geophyllum*
13.	*EM- 1036*	*Cenococcum geophyllum*
14.	*EM- 1035*	*Cenococcum geophyllum*
15.	*EM- 1150*	*Cenococcum geophyllum*
16.	EM- 1149	Cenococcum geophyllum
17.	*EM- 1252*	*Cenococcum geophyllum*
18.	*EM- 1088*	*Cenococcum geophyllum*
19.	*EM- 1253*	*Chalciporus piperatus*
20.	*EM- 1153*	*Ectendomycorrhizae*
21.	*EM- 1154*	*Ectendomycorrhizae*
22.	*EM- 1155*	*Ectendomycorrhizae*
23.	*EM- 1157*	*Elaphomyces granulatus*
24.	*EM- 1156*	*Elaphomyces granulatus*
25.	*EM- 1108*	*Gautieria otthii*
26.	*EM- 1138*	*Gautieria caudata*
27.	*EM- 1158*	*Gyrodon lividus*
28.	*EM- 1163*	*Hebeloma crustuliniforme*
29.	*EM- 1160*	*Hebeloma circinans*
30.	*EM- 1159*	*Hebeloma calyptosporum*
31.	*EM- 1164*	*Hebeloma crustuliniforme*
32.	*EM- 1171*	*Hebeloma crustuliniforme*
33.	*EM- 1172*	*Hebeloma cylindrosporum*
34.	*EM- 1173*	*Hebeloma cylindrosporum*
35.	*EM- 1170*	*Hebeloma crustuliniforme*
36.	EM- 1169	Hebeloma crustuliniforme
37.	*EM- 1174*	*Hebeloma edurum*
38.	*EM- 1168*	*Hebeloma crustuliniforme*
39.	*EM- 1165*	*Hebeloma crustuliniforme*
40.	*EM- 1175*	*Hebeloma edurum*
41.	*EM- 1008*	*Hebeloma crustuliniforme*
42.	*EM- 1180*	*Hebeloma sinapizans*
43.	*EM- 1037*	*Cenococcum geophyllum*
44.	*EM- 1015*	*Hebeloma crustuliniforme*
45.	*EM- 1183*	*Hebeloma truncatum*
46.	*EM- 1182*	*Hebeloma sinapizans*
47.	*EM- 1184*	*Hebeloma vaccinum*
48.	*EM- 1176*	*Hebeloma inagratum*
49.	*EM- 1177*	*Hebeloma mesophaeum*
50.	*EM- 1185*	*Hysterangium incarceratum*
51.	*EM- 1263*	*Lactarius quietus*
52.	*EM- 1122*	*Laccaria farinacea*
53.	*EM- 1103*	*Laccaria bicolor*
54.	*EM- 1105*	*Laccaria laccata*
55.	*EM- 1186*	*Laccaria amethystina*
56.	EM- 1079	Laccaria laccata
57.	*EM- 1076*	*Laccaria laccata*
58.	*EM-1083*	*Laccaria fraterna*
59.	*EM- 1086*	*Laccaria proxima*
60.	*EM- 1085*	*Laccaria laccata*
61.	*EM- 1187*	*Laccaria bicolor*
62.	*EM- 1188*	*Laccaria laccata*
63.	*EM- 1193*	*Laccaria laccata*
64.	*EM- 1196*	*Laccaria tortilis*
65.	*EM- 1195*	*Laccaria proxima*
66.	*EM- 1102*	*Laccaria bicolor*
67.	*EM- 1192*	*Laccaria laccata*
68.	*EM- 1189*	*Laccaria laccata*
69.	*EM- 1190*	*Laccaria laccata*
70.	*EM- 1191*	*Laccaria laccata*
71.	*EM- 1104*	*Laccaria laccata*
72.	*EM- 1091*	*Laccaria amethystina*
73.	*EM- 1058*	*Laccaria laccata*
74.	*EM- 1032*	*Laccaria laccata*

S.No.	Bank Code	Culture Name
75.	*EM- 1033*	*Laccaria laccata*
76.	EM- 1042	Laccaria laccata
77.	*EM- 1275*	*Laccaria proxima*
78.	*EM- 1066*	*Laccaria laccata*
79.	*EM- 1031*	*Laccaria laccata*
80.	*EM- 1067*	*Laccaria laccata*
81.	*EM- 1065*	*Laccaria laccata*
82.	*EM- 1090*	*Laccaria laccata*
83.	*EM- 1009*	*Laccaria laccata*
84.	*EM- 1207*	*Laccaria scaber*
85.	*EM- 1207A*	*Laccaria scaber*
86.	*EM- 1261*	*Laccaria deliciosus*
87.	*EM- 1279*	*Laccaria hepaticus*
88.	*EM- 1264*	*Laccaria rufus*
89.	*EM- 1052*	*Laccaria rufus*
90.	*EM- 1260*	*Laccaria chrysorrheus*
91.	*EM- 1262*	*Laccaria deterrimus*
92.	*EM- 1206*	*Laccaria tabidus*
93.	*EM- 1199*	*Laccaria deterrimus*
94.	EM- 1198	Laccaria controversus
95.	*EM- 1197*	*Laccaria controversus*
96.	*EM- 1202*	*Laccaria rufus*
97.	*EM- 1204*	*Laccaria subdulcis*
98.	*EM- 1259*	*Laccaria chrysornheus*
99.	*EM- 1205*	*Laccaria subdulcis*
100.	*EM- 1203*	*Laccaria rufus*
101.	*EM- 1133*	*Leccinum scabrum*
102.	*EM- 1106*	*Leccinum insigne*
103.	*EM- 1281*	*Leccinum aurantiacum*
104.	*EM- 1113*	*Martellia ellipsospora*
105.	*EM- 1130*	*Melanogaster tuberiformis*
106.	*EM- 1125*	*Melanogaster tuberiformis*
107.	*EM- 1131*	*Melanogaster varigatus*
108.	*EM- 1134*	*Paxillus involutus*
109.	*EM- 1209*	*Paxillus involutus*
110.	*EM- 1266*	*Paxillus involutus*
111.	*EM- 1270*	*Paxillus involutus*
112.	*EM- 1267*	*Paxillus involutus*
113.	*EM- 1268*	*Paxillus involutus*
114.	EM- 1269	Paxillus involutus
115.	*EM- 1073*	*Paxillus involutus*
116.	*EM- 1208*	*Paxillus involutus*
117.	*EM- 1141*	*Paxillus involutus*
118.	*EM- 1217*	*Paxillus involutus*
119.	*EM- 1212*	*Paxillus involutus*
120.	*EM- 1282*	*Paxillus involutus*
121.	*EM- 1055*	*Phaeolepiota aurea*
122.	*EM- 1047*	*Phaeolepiota fortinii*
123.	*EM- 1219*	*Piloderma*
124.	*EM- 1002*	*Pisolithus tinctorius*
125.	*EM- 1057*	*Pisolithus tinctorius*
126.	*EM- 1010*	*Pisolithus tinctorius*
127.	*EM- 1081*	*Pisolithus tinctorius*
128.	*EM- 1059*	*Pisolithus tinctorius*
129.	*EM- 1005*	*Pisolithus tinctorius*
130.	*EM- 1006*	*Pisolithus tinctorius*
131.	*EM- 1034*	*Pisolithus tinctorius*
132.	*EM- 1271*	*Pisolithus tinctorius*
133.	EM- 1221	Pisolithus tinctorius
134.	*EM- 1223*	*Pisolithus tinctorius*
135.	*EM- 1224*	*Pisolithus tinctorius*
136.	*EM- 1004*	*Pisolithus tinctorius*
137.	*EM- 1022*	*Rhizopogon subareolatus*
138.	*EM- 1019*	*Rhizopogon fuscorubens*
139.	*EM- 1025*	*Rhizopogon vulgaris*
140.	*EM- 1043*	*Rhizopogon smithii*
141.	*EM- 1040*	*Rhizopogon mutabilis*
142.	*EM- 1039*	*Rhizopogon vulgaris*
143.	*EM- 1029*	*Rhizopogon cusickensis*
144.	*EM- 1024*	*Rhizopogon subscaerulescens*
145.	*EM- 1023*	*Rhizopogon vulgaris*
146.	*EM- 1013*	*Rhizopogon arenicola*
147.	*EM- 1044*	*Rhizopogon colossus*
148.	*EM- 1038*	*Rhizopogon ellenae*
149.	*EM- 1017*	*Rhizopogon occiden*
150.	*EM- 1049*	*Rhizopogon rubescens*
151.	*EM- 1053*	*Rhizopogon parksii*
152.	EM- 1048	Rhizopogon subscaerulescens
153.	*EM- 1054*	*Rhizopogon vulgaris*
154.	*EM- 1014*	*Rhizopogon vinicolor*
155.	*EM- 1050*	*Rhizopogon rubescens*
156.	*EM- 1056*	*Rhizopogon villosulus*

S.No.	Bank Code	Culture Name
157.	*EM- 1028*	*Rhizopogon clavitisporus*
158.	*EM- 1027*	*Rhizopogon hawkerae*
159.	*EM- 1007*	*Rhizopogon subscaerulescens var. subpannosus*
160.	*EM- 1026*	*Rhizopogon ochraceorubens*
161.	*EM- 1018*	*Rhizopogon subscaerulescens*
162.	*EM- 1118*	*Rhizopogon vinicolor*
163.	*EM- 1227*	*Rhizopogon luteolus*
164.	*EM- 1226*	*Rhizopogon luteolus*
165.	*EM- 1128*	*Rhizopogon smithii*
166.	*EM- 1127*	*Rhizopogon reaii*
167.	*EM- 1228*	*Rhizopogon nigrescens*
168.	*EM- 1229*	*Rhizopogon roseolus*
169.	*EM- 1134*	*Rhizopogon evadens*
170.	*EM- 1232*	*Rhizopogon vulgaris*
171.	*EM- 1231*	*Rhizopogon subrerolatus*
172.	EM- 1230	Rhizopogon rubescens
173.	*EM- 1012*	*Rhizopogon ochraceorubens*
174.	*EM- 1119*	*Rhizopogon occidentalis*
175.	*EM- 1046*	*Rhizopogon ellenae*
176.	*EM- 1063*	*Rhizopogon vulgaris*
177.	*EM- 1061*	*Rhizopogon rubescens*
178.	*EM- 1011*	*Rhizopogon parksii*
179.	*EM- 1109*	*Rhizopogon rubescens*
180.	*EM- 1110*	*Rhizopogon vinicolor*
181.	*EM- 1116*	*Rhizopogon vulgaris*
182.	*EM- 1115*	*Rhizopogon ochraceorubens*
183.	*EM- 1114*	*Rhizopogon hawkerae*
184.	*EM- 1112*	*Rhizopogon smithii*
185.	*EM- 1135*	*Rhizopogon ellenae*
186.	*EM- 1126*	*Rhizopogon fuscorubens*
187.	*EM- 1140*	*Rhizopogon ochraceisporus*
188.	*EM- 1142*	*Rhizopogon rubescens*
189.	*EM- 1143*	*Rhizopogon vinicolor*
190.	*EM- 1132*	*Sarcodon scabrosus*
191.	EM- 1273	Scleroderma aurantium
192.	*EM- 1235*	*Scleroderma flavidum*
193.	*EM- 1233*	*Scleroderma cepa*
194.	*EM- 1107*	*Scleroderma citrinum*
195.	*EM- 1240*	*Suillus granulatus*
196.	*EM- 1241*	*Suillus granulatus*

S.No.	Bank Code	Culture Name
197.	*EM- 1030*	*Suillus americanus*
198.	*EM- 1075*	*Suillus variegatus*
199.	*EM- 1244*	*Suillus luteus*
200.	*EM- 1243*	*Suillus luteus*
201.	*EM- 1245*	*Suillus variegatus*
202.	*EM- 1074*	*Suillus bovinus*
203.	*EM- 1239*	*Suillus bovinus*
204.	*EM- 1246*	*Suillus variegatus*
205.	*EM- 1071*	*Suillus luteous*
206.	*EM- 1111*	*Suillus lakei*
207.	*EM- 1123*	*Suillus tomentosus*
208.	*EM- 1121*	*Suillus brevipes*
209.	*EM- 1124*	*Suillus brevipes*
210.	EM- 1051	Suillus tomentosus
211.	*EM- 1136*	*Suillus luteus*
212.	*EM- 1137*	*Suillus luteus*
213.	*EM- 1120*	*Suillus granulatus*
214.	*EM- 1087*	*Suillus subluteous*
215.	*EM- 1237*	*Suillus bellini*
216.	*EM- 1238*	*Suillus boviniodes*
217.	*EM- 1021*	*Suillus tomentosus*
218.	*EM- 1117*	*Suillus punctatipes*
219.	*EM- 1236*	*Suillus bellini*
220.	*EM- 1247*	*Thelephora terrestris*
221.	*EM- 1077*	*Thelephora terrestris*
222.	*EM- 1078*	*Thelephora terrestris*
223.	*EM- 1062*	*Thelephora terrestris*
224.	*EM- 1249*	*Tricholoma populinum*
225.	*EM- 1248*	*Tricholoma albobruneum*
226.	*EM- 1276*	*Tricholoma ustale*
227.	*EM- 1250*	*Tricholoma scalpturatum*
228.	*EM- 1041*	*Tuber melanosporum*
229.	*EM- 1144*	*Wilcoxinia mikolae*
230.	EM-1310	*Boletus sp.*
231.	*EM- 1288*	*Geastrum sp.*
232.	*EM- 1311*	*Hysterangium sp.*
233.	*EM- 1285*	*Lycoperdon sp.*
234.	*EM- 1290*	*Pisolithus tinctorius*
235.	*EM- 1291*	*Pisolithus tinctorius*
236.	*EM- 1286*	*Russula Sp.*
237.	*EM- 1292*	*Scleroderma verucosum*

S.No.	Bank Code	Culture Name
238.	*EM- 1287*	*Suillus sp.*
239.	*EM- 1310*	*Rizopogon luteolus*
240.	*EM- 1284*	*Scleroderma cepa*
241.	*EM- 1283*	*Scleroderma verucosum*
242.	*EM- 1293*	*Pisolithus tinctorius*
243.	*EM- 1289*	*Pisolithus tinctorius*
244.	EM- 1161	*Hebeloma crustuliniformi*

S.No.	Bank Code	Culture Name
245.	EM- 1312	*Lycoperdon*
246.	EM- 1298	*Pisolithus tinctorius* Almora
247.	EM- 1299	*Pisolithus tinctorius* Korba
248.	EM- 1300	*Pisolithus tinctorius* Pachmarhi
249.	EM- 1301	*Pisolithus tinctorius* Faridabad
258.	EM- 1310	*Pisolithus tinctorius* Bhopal

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:

- *Applied Soil Ecology*
- *Biosystems*
- *Chemosphere*
- *Crop Protection*
- *Environmental Pollution*
- *European Journal of Soil Biology*
- *Fungal Ecology*
- *Journal of Environmental Radioactivity*
- *Mycoscience*
- *Physiological and Molecular Plant Pathology*
- *Plant Physiology and Biochemistry*
- *Plant Science*
- *Review of Palaeobotany and Palynology*
- *Science of The Total Environment*
- *Scientia Horticulturae*
- *Soil Biology and Biochemistry*
- *Trends in Plant Science*

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