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Impact of Elevated CO_2 and/or Temperature on the AM Fungal Diversity in Groundnut Rhizosphere under Open Top Chamber facility

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Investigations were carried to study the impact of elevated carbon dioxide and temperature on Arbascular Mychorhizal fungal (AMF) community in groundnut crop under the open top chamber facility at Institute for Agricultural Research on Climate Change, University of Agricultural Sciences, Dharwad. The results of the investigations have revealed that the Shannon diversity index was highest with elevated CO_2 plus temperature (H'=3.10), while elevated CO_2 recorded (H'=2.25). The Margalef's index of species richness for AMF was highest in elevated CO_2 plus temperature (2.66) compared to ambient condition (1.37). Further, the most predominate species *Glomus mossae* from the five altered conditions were genetically authenticated by amplifying at 375 bp specific DNA band in the ITS region of AM fungi.

Introduction

Enhancement in concentration of atmospheric carbon dioxide (CO_2) and resultant increasing temperature as a result of the cumulative effects of anthropogenic activities, emissions from fossil fuel combustion, energy use scenario, land clearing and urbanization have created renewed interest in understanding responses of the ecosystem to increased levels of CO_2 and temperature (IPCC, 2007). The atmospheric CO_2 concentration has been steadily rising from pre-industrial era: 280 ppm in the year 1850 to 390 ppm in 2011. As a consequence of this rise in CO_2, the global surface temperature is predicted to increase by 1 to 6º C by the year 2100, leading to enhanced drought in several areas of the world, as a result of increased evapo-transpiration.

These climate-changing parameters are known to affect terrestrial plants as well as large varieties of microorganisms which are associated with the host plants; thereby enhancing the plant growth promotion as well as increasing the plant tolerance to biotic and abiotic stresses. Many of these plant growth-promoting microorganisms colonize the rhizosphere soil, the portion of soil attached

to the root surface and influenced by root exudates. Some of the beneficial microorganisms can also enter the root system of their hosts and enhance their beneficial effects with an endophytic lifestyle like plant growth-promoting fungi (PGPF) such as arbuscular mycorrhizal fungi (AMF) as well as plant growth-promoting rhizobacteria.

Among the beneficial soil microorganisms, approximately 90 per cent of the plants form mycorrhizal association and 60 per cent of these plants establish symbiosis with obligate symbionts such as AMF and are known to enhance plant nutrient uptake in exchange for rhizosphere soil carbohydrate compounds. However, due to climate change effects including increasing CO_2 concentrations in the atmosphere, soil warming or drought stress has indirect effect on plant associated AMF (Auge, 2001). Several studies have revealed that elevated CO_2 increased the AMF colonization in comparison with ambient CO_2, due to increased carbon allocation to their external hyphae. However, contrasting results also have been obtained by Gavito *et al*. (2000) who indicated that certain AMF strains did not respond to altered conditions. Hence, different plant growth promoting strains need to be selected for elevated CO_2 and temperature conditions.

In order to overcome, as well as to address the global warming effect on the beneficial microbes, there is a need to study the effect of elevated CO_2 and temperature on beneficial microorganisms. In this regard, an approach has been made to study the effect of elevated CO_2, elevated temperature and combination of elevated CO_2 along with increased temperature on soil biological parameters with a special emphasis on AM fungal community in groundnut rhizosphere soil. This will form a basis for the development of AMF consortium under altered or to be altered climatic conditions. An attempt will also be made to study the genetic variability of the predominant group of the AMF under altered conditions by amplifying the internal transcribed spacer of the ribosomal DNA (ITS rDNA).

Materials and Methods

An investigation was carried out to study the influence of elevated carbon dioxide, temperature and their combinations on the abundance of the AMF community in groundnut rhizosphere soil as well as to study the genetic variability of the predominant AMF species in the Open Top Chamber (OTC) facility at the Institute for Agricultural Research on Climate Change (IARCC), University of Agricultural Sciences, Dharwad located at 15^0 29′ 44.7″ N latitude ; 74^0 59′ 13.1″ E longitude, and at an altitude of 678 m above mean sea level.

Soil sampling: Each OTC was considered as a treatment for this study, which included T_1 (Elevated carbon dioxide upto 550 ppm + elevated temperature of +2^0C), T_2 (Elevated carbon dioxide 550 ppm), T_3 (Elevated temperature +2^0C), T_4 (Ambient condition or only chamber effect), T_5 (Reference plot or Open ring). The groundnut, cultivar GPBD-4, as test crop was sown and grown during kharif 2013 inside the OTC. Rhizosphere soil samples from 0 to 15 cm depth were collected at the flowering stage of the crop to study the AM fungal diversity.

Arbuscular Mycorrhizal spore count

The chlamydospores in groundnut rhizosphere soil of groundnut were determined by using wet sieving and decantation method as outlined by Gerdemann and Nicholson (1963). Hundred gram of soil sample was taken in one liter beaker, made up into 1000 ml with water, stirred well, heavier soil particles were

allowed to settle for a few seconds. Then the suspension was passed through a series of different size sieves (250µm, 106µm, 75µm, 45µm, 37µm) arranged in the descending order of their mesh size. Again water was added up to 1000 ml, stirred well and allowed for few seconds this was repeated for five to six times, till the suspension appeared clear. Sievates were collected from each sieve separately in beakers. The supernatant from each beaker was separately filtered through Whatman No. 1 filter paper and the content of the filter papers were examined for spores under stereo zoom microscope (LABOMED).

Arbuscular Mycorrhizal community study

Trap culture technique: The test soil sample was mixed with equal amount of sterilized sand soil mixture (1:1) and suitable host plants (mixture of sorghum and cow pea) were sown. The plants were watered twice weekly. Hoglands nutrient solution was added bi-weekly. After 3-4 months, the potting mix was wet sieved and the spores were counted under a stereomicroscope and grouped according to their morphological characteristics. The richness and relative abundance of each fungal type were calculated.

Extraction of AM spores from the soil: AM fungal spore extraction was done as outlined by Gerdemann and Nicholson (1963). Further, the collected sieving was subjected to the sucrose density gradient centrifugation method which avoids the debris remains with the spores. The suspension was passed through 37 µm sieve and AMF spore samples were collected by washing with distilled water and filtered through Whatman No.1 filtered paper.

Spore identification: Morphologically similar spores were picked and the population of each spore type was enumerated. Single spore from each morphtypes was brought in to funnel technique after surface sterilization of spore with 200 ppm streptomycin sulphate and 2% chloramineT. Once roots start emerging from the stem-tips of the funnels, the contents are transferred to small plastic cups containing sterilized sand: soil (1:1) after checking the roots for AM colonization. The spores were mounted on a clean glass slide in lacto phenol. The spore identification was mainly based on morphological features, *viz*., colour, size, wall structure and hyphal attachment according to the outline given by Schenk and Perez. Finally, the spores were photographed under Olympus research stereomicroscope connected to a computer with digital image analysis software.

Diversity analysis: Margalef's Species richness index. The Margalef's species richness index is the simplest and can be computed based on the formula:

Margalef's species richness index = DMg = (S-1) / ln N

Where,
ln = Natural Log i.e., $\log_e$
S = Total number of species
N = Total number of individuals summed over all species
Higher the value of the index, higher is the diversity.

Shannon-Wiener index: The Shannon's index has probably been the most widely used index in community ecology. It is a measure of the average degree of "uncertainty" in predicting to what species an individual chosen at random from a collection of S species and N individuals will belong.

Shannon's index is estimated by using formula

$$H' = -\sum^{S} [(n_i/N) \ln (n_i/N)]$$

i=1
Where,

n_i = Number of individuals belonging to the ith species
N = Total number of individuals in the sample

The quantity P_i is the proportion of individuals found in the ith species. The higher the value of index, higher is the diversity.

Per cent colonization: Mycorrhizal root colonization was determined as per the procedure proposed by Philips and Hayman (1970). Fresh root samples were cut into 1 cm pieces and placed in screw cap vials. The clearing of the roots was achieved by treating them with 10 percent KOH and leaving them overnight. The KOH solution was poured off and the roots were rinsed with tap water. Later, the roots were treated with 10 per cent HCl for 10 minutes to neutralize the residual effect of alkali and create an acidic environment required for further staining. The root bits were stained with 0.05 per cent trypan blue in lactoglycerol (lactic acid, glycerol and water in the ratio of (40:20:20 respectively) by boiling them at 90º C for 30 minutes. Excess stain was decanted and the root samples were immersed in lactoglycerol for destaining. The stained root bits were placed on a clean glass slide and observed under microscope for colonization. The percentage of roots colonized by mycorrhizae was calculated by the formula:

$$\text{Per cent root colonization} = \frac{\text{Root bits positive for colonization}}{\text{Total number of root bits}} \times 100$$

Genetic characterization of AMF: The most predominant species of AMF from five altered environmental condition were selected. Gene sequences were amplified by using Primer Glo ITS-I 5'-AAGCGAGTCGAGCGTTAAGC-3' Antisense primer Glo ITS-II 5'-ACCGGATGGATGAATTTC-3'. The primer synthesis was done by SIGMA- ALDRICH.

Amplification of genes from AMF spores: For the PCR amplification, a single surface sterilized spore of AMF was placed in a sterile PCR vial. The presence of single spore in the PCR vial was confirmed by observing under stereomicroscope and then subjected for a short spin. The vial containing spore was kept in water bath at 95^0 C for 10 min. PCR reaction mixture containing 0.1 mM dNTPs, 5 pM was made each of primer, 5 U of *Taq* DNA polymerase and the supplied reaction buffer amounting a total volume of 20 µl was made. The reactions were carried out in a polymerase chain reaction (PCR) machine (Eppendorf). The reaction mixture was cycled through the following temperature profile: 94^0 C for 5 min; 94°C for 1 min; 54^0 C for 1 min; 72^0 C for 45 min and holding at 4^0 C. Further, The PCR samples were subjected to gel electrophoresis (BIO-RAD) using 1.5 per cent agarose gel along with 100 bp molecular marker of λ DNA. Gene amplification was observed under UV transilluminator and documented.

Results and Discussion

Influence of elevated CO_2 and/ or temperature on Mycorrhizal parameters

AM fungal spore count as influenced by elevated CO_2 and/ or temperature in groundnut rhizosphere soil

Elevated CO_2 often increases mycorrhizal colonization of plant roots, since elevated

CO_2 enhances C allocation to roots. Further, AM fungi rely on host plant carbon and mycorrhizal colonization increases sink strength of the root. Our experiment revealed that the AM fungal spore count as well as root colonization was significantly influenced by elevated CO_2 condition as compared to other altered conditions.

A positive feedback loop can be envisaged, in which plants respond to elevated CO_2 by increased C fixation followed by the transfer of more carbon to their root systems. Consequently, mycorrhizal fungi might grow more and capture more phosphate (Maria *et al*., 2008).

AM fungal community as influenced by elevated CO_2 and/ or temperature

In this study, as many as 39 AM fungal morphotypes belonging to four genera were isolated and tentatively identified. The highest number of native AMF species was recorded with elevated CO_2 plus temperature (20.00), followed by reference plot (14), elevated CO_2 (13), elevated temperature (11) and the lowest number of species was recorded under ambient condition (9). Similar variation in AMF species composition has been reported by Anita *et al*. (2011).

Abundance of AM fungal spores of different species as influenced by elevated CO_2 and/ or temperature condition.

AM fungal abundance was measured by enumerating the spore density in rhizosphere soil the soil sample. In the present investigation, spores of *Glomus fasciculatum* were the most abundant under elevated CO_2 plus temperature condition (35 spores 50 g^{-1}soil) and also under elevated CO_2 condition (37 spores 50 g^{-1} soil). While Glomus *mossae* was the most abundant type under elevated temperature, ambient and in open reference ring (26; 28 and 33 spores 50 g^{-1}soil respectively). Anita *et al.* (2011) recorded three significant indicator species of elevated CO_2 belonging to *Glomus microaggregatum; Glomus constrictum* and *Entrophospora. infrequens* compared to ambient condition, indicating the diverse response of mycorrhizal strains towards altered conditions. Suggesting that AMF belonging to *Glomus* are likely to be more abundant under elevated environmental condition

Diversity of AMF as influenced by elevated CO_2 and/ or temperature based on spore count

In the present study, variation in Shannon diversity index of AM fungal species in altered environmental conditions viz., elevated CO_2 plus temperature, elevated CO_2, elevated temperature, ambient condition and reference plot was observed based on the spore load.

The Shannon diversity index of AM fungi was more in elevated CO_2 plus temperature condition (3.10), followed by reference plot (2.45), and elevated temperature condition (2.37) based on the spore count. The altered environmental conditions positively influence AMF diversity.

In the present investigation, it was observed that there was a clear difference in the AM fungal species richness between the altered conditions. The Margalef species richness was greater under the influence of elevated CO_2 and temperature (2.66) followed by reference plot (1.86) and the lowest species richness was recorded in ambient condition (1.37). Similar variation in AMF species composition has been reported by Anita *et al*. (2011). Combination of elevated CO_2 plus temperature condition could promote specific AM fungal groups reflecting in species richness.

Table.1 Mycorrhizal spore count as influenced by elevated CO2 and/ or temperature in groundnut rhizosphere

Treatments	**Mycorrhizal spore count (Number of spore 50 g^{-1})**	
	30 DAS	**60 DAS**
Elevated CO_2 +Temp	224.75	330.00
Elevated CO_2	227.00	336.50
Elevated Temperature	223.25	328.75
Ambient	221.50	324.75
Reference ring	219.50	318.75
S.Em±	**1.29**	**2.09**
CD (0.05)	**3.88**	**6.31**

Table.2 Mycorrhizal root colonization as influenced by elevated CO2 and/ or temperature in groundnut rhizosphere

Treatments	**Percent root colonization**
Elevated CO_2 +Temp	54.00
Elevated CO_2	55.75
Elevated Temperature	51.50
Ambient	50.50
Reference ring	47.00
SEm±	**0.50**
CD (0.05)	**1.49**

Table.3 Species of AM fungi present in five altered conditions of OTC

Sl No	**Elevated CO_2 + temperature**	**Elevated CO_2**	**Elevated temperature**	**Ambient conditions**	**Reference plot**
1	*A. appendicula*	*A.lacunosa*	*A.mellea*	*A.lacunosa*	*A.dilatata*
2	*A. lacunose*	*Acaulospora*	*A.rugosa*	*A.morrowae*	*A.lacunosa*
3	*A. longula*	*Gigaspora*	*A.scrobiculata*	*A.scrobiculata*	*A.morrowe*
4	*A. marrowe*	*G.diaphanum*	*A.spinosa*	*G.etunicatum*	*A.sporocarpum*
5	*Acaulospora*	*G.etunicatum*	*Acaulospora spp*	*G.fasciculatum*	*Acaulospora spp*
6	*G. ambisporum*	*G.farmosanum*	*G.fasciculatum*	*G.lacteum*	*E. singulae*
7	*G. constrictum*	*G.fasciculatum*	*G.lacteus*	*G.multicaulis*	*G.ambispora*
8	*G. etunicatum*	*G.foevata*	*G.macrcarpum*	*G.mosseae*	*G.farmosanum*
9	*G.fasciculatum*	*G.lacteum*	*G.mosseae*	*G.versiformae*	*G. fasciculatum*
10	*G.gerdimanii*	*G.macrcarpum*	*G.radiata*		*G.macrocarpum*
11	*G.geosporum*	*G.mosseae*	*Glomus sp.*		*G. mosseae*
12	*G. globifrum*	*Glomus*			*G.multicaulis*
13	*G. leptotichum*	*S.heterogama*			*G. occultum*
14	*G. macrocarpum*				*G.radiatum*
15	*G. mosseae*				
16	*G. pubescens*				
17	*Glomus radiata*				
18	*G. radiates*				
19	*Glomus spp*				
20	*Scutellospora*				
Total	**20**	**13**	**11**	**9**	**14**

Note: A – *Acaulospora*, G- *Glomus*, S – *Scutellospora*, E- *Entrophospora*

Table.4 Abundance of spores of different species of AM fungi in five altered condition of OTC

Sl. No	AM fungal species	Mycorrhizal spore load (No. of spores per 50 g of soil)				
		Elevated CO_2+temperature	Elevated CO_2	Elevated temperature	Ambient condition	Reference plot
1	*A. appendicula*	20	-	-	-	-
2	*A.dilatata*	-	-	-	-	15
3	*A. lacunose*	25	21	-	18	16
4	*A. longula*	23	-	-	-	-
5	*A. marrowe*	18	-	-	23	21
6	*A.mellea*	-	-	17	-	-
7	*A.rugosa*	-	-	12	-	-
8	*A.scrobiculata*	-	-	16	14	-
9	*A.spinosa*	-	-	18	-	-
10	*A.sporocarpum*	-	-	-	-	19
11	*Acaulospora spp*	21	18	23	-	21
12	*E. singulae*	-	-	-	-	12
13	*G. ambisporum*	23	-	-	-	-
14	*G.diaphanum*	-	24	-	-	-
15	*G. constrictum*	16	-	-	-	-
16	*G. etunicatum*	22	15	-	18	-
17	*G.farmosanum*	-	12	-	-	14
18	*G.fasciculatum*	35	37	-	16	18

19	***G.foevata***	-	17	-	-	-
20	***G.gerdimanii***	19	-	-	-	-
21	***G.geosporum***	15	-	-	-	-
22	***G. globifrum***	17	-	-	-	-
23	***G.infrequens***	21	-	-	-	-
24	***G.lacteum***	-	26	-	20	-
25	***G.lacteus***	-	-	15	-	-
26	***G. leptotichum***	12	-	-	-	-
27	***G. macrocarpum***	25	23	-	-	21
28	***G. mosseae***	32	35	26	28	33
29	***G.multicaulis***	-	-	-	15	17
30	***G. occultum***	-	-	-	-	13
31	***G. pubescens***	23	-	-	-	-
32	***G.radiata***	26	-	19	-	-
33	***G. radiates***	21	-	-	-	-
34	***G.radiatum***	15	-	-	-	-
34	***G.versiformae***	24	-	-	23	-
36	***Glomus sp.***	26	-	22	-	-
37	***Gigaspora***	-	-	18	15	-
38	***S.heterogama***	-	-	11	-	-
39	***Scutellospora***	14	-	-	-	-
	Total	**493**	**228**	**197**	**190**	**220**

Table.5 Tentative identification of AM fungal native morphotypes from five altered environmental condition

Sl No.	Isolates	Spore Size (µm)	Colour	Spore wall size(µm)	Spore surface	Hyphal length (µm)	Probable species identified
1	AMF A1	99.5	Yellow	3.4	Smooth	-	*Acaulospora*
2	AMF A2	210.2	Brown	3.7	Rough	-	*Acaulospora*
3	AMF A3	138.4	Brown	3.5	Rough	-	*Acaulospora*
4	AMF A4	148.5	Yellow	3.4	Rough	67.6	*Glomus*
5	AMF A5	135.3	Yellow	3.2	Smooth	-	*Glomus constrictum*
6	AMF A6	110.4	Brown	3.3	Rough	30.66	*Glomus radiata*
7	AMF A7	184.9	Yellow	3.7	Smooth	-	*Glomus etunicatum*
8	AMF A7 (a)	117.8	Yellow	3.4	Smooth	90.3	*G. leptotichum*
9	AMF A8	171.2	Brown	3.1	Smooth	74.2	*G. globifrum*
10	AMF A9	135.4	Yellow	3.6	Rough	120.3	*A. longula*
11	AMF A10	148.4	Dark brown	3.8	Smooth		*G.geosporum*
12	AMF A11	308.4	Yellow	3.3	Smooth	-	*A. appendicula*
13	AMF A12	164.3	Black	3.2	Rough	-	*G.infrequens*
14	AMF A13	148.4	Yellow	4.3	Rough and granular	70.8	*A. lacunose*
15	AMF A14	110.6	Yellow	3.4	Smooth	67.3	*G. fasciculatum*
16	AMF A15	180.1	Yellow	3.9	Rough	-	*G. pubescens*
17	AMF A16	152.6	Yellow	3.2	Rough	52.4	*Scutellospora*
18	AMF A17	185.3	Black	3.4	Smooth	-	*Glomus*
19	AMF A18	184.1	Brown	3.6	Smooth	29.3	*G.gerdimanii*
20	AMF A19	214.3	Yellow	4.3	Rough	56.8	*G. macrocarpum*
21	AMF A20	176.4	Black	-	Smooth	-	*G. ambisporum*
22	AMF A21	91.1	Dark brown	2.2	Smooth	-	*Glomus*
23	AMF A23	86.3	Yellow	3.2	Smooth	-	*G. radiates*
24	AMF A24	143.6	Yellow	4.1	Rough	-	*G.fasciculatum*
25	AMF A25	92.3	Black	-	Smooth	-	*G. ambisporu*
26	AMF A28	118.5	Yellow	4.2	Smooth	43.6	*G. fasciculatum*
27	AMF A29	143.3	Brown	4.4	Smooth		*Glomus*

28	AMF A32	157.8	Yellow	3.5	Rough	-	*A. marrowe*
29	AMF C1	153.2	Yellow	3.6	Smooth	-	*G.etunicatum*
30	AMF C3	163.9	Yellow	5.7	Rough	-	*G. lacteum*
31	AMF C4	157.8	Brown	-	Rough	-	*Sporocarp*
32	AMF C5	107.5	Yellow	5.2	Rough	-	*Glomus*
33	AMF C6	163.7	Yellow	4.6	Rough and granular	43.5	*G. mosseae*
34	AMF C7	167.4	Brown	4.3	Rough and granular	-	*G.farmosanum*
35	AMF C9	183.2	Yellow	3.7	Rough and granular	45.2	*Gigaspora*
36	AMF C10	108.3	Yellow	5.4	Smooth	-	*G.fasciculatum*
37	AMF C13	162.3	Yellow	3.4	Rough	35.6	*Gigaspora*
38	AMF C14	208.3	Yellow	3.7	Rough	-	*A.lacunosa*
39	AMF C15	154.7	Brown	7.2	Smooth	49.72	*Acaulospora*
40	AMF C16	203.4	Yellow	5.3	Smooth	-	*G.lacteum*
41	AMF C17	152.7	Yellow	5.7	Rough	52.7	*G.foevata*
42	AMF C18	112.8	Yellow	3.7	Rough and granular	67.4	*Scutellospora heterogama*
43	AMF C20	103.5	Brown	6.4	Smooth	31.9	*G.mosseae*
44	AMF C21	88.7	Yellow	3.6	Smooth	-	*G.diaphanum*
45	AMF C22	94.7	Yellow	6.7	Rough		*G.macrcarpum*
46	AMF C23	99.8	Yellow	5.2	Smooth	-	*G.mosseae*
47	AMF C24	104.4	Yellow	7.1	Rough	-	*Glomus*
48	AMF C25	80.9	Yellow	4.6	Smooth	-	*G.fasciculatum*
49	AMF C26	116.3	Yellow	7.1	Smooth	57.9	*G.fasciculatum*
50	AMF C28	81.3	Yellow	3.6	Smooth	-	*G.lacteum*
51	AMF C29	94.8	Brown	5.3	Smooth	-	*G.fasciculatum*
52	AMF C31	99.2	Brown	2.7	Smooth	-	*G.fasciculatum*
53	AMF D2	171.6	Yellow	4.4	Smooth	-	*Glomus sp.*
54	AMF D3	193.5	Yellow	3.4	Smooth	-	*A.rugosa*
55	AMF D4	147.3	Brown	7.3	Smooth	22.2	*G.radiata*
56	AMF D6	181.4	Yellow	4.8	Rough and granular	-	*A.scrobiculata*
57	AMF D8	139.6	Yellow	3.8	Smooth	-	*A.spinosa*
58	AMF D9	132.1	Yellow	5.4	Rough and granular	-	*A.spinosa*
59	AMF D12(d)	135.6	Yellow	8.3	Rough and granular	-	*A.scrobiculata*

60	AMF D13(b)	109.3	Yellow	7.1	Rough and granular	-	*A.scrobiculata*
61	AMF D14(a)	159.1	Yellow	3.8	Rough and granular	-	*G.lacteus*
62	AMF D14(b)	132.4	Yellow	4.5	Smooth	-	*A.spinosa*
63	AMF D14(d)	125.7	Yellow	3.6	Rough and granular	-	*A.mellea*
64	AMF D14(e)	124.8	Yellow	4.1	Rough and granular	-	*Acaulospora*
65	AMF D15	90.5	Yellow	3.2	Rough and granular	-	*Acaulospora*
66	AMF D16(a)	158.1	Yellow	6.5	Rough and granular	-	*Acaulospora*
67	AMF D16(b)	120.6	Yellow	4.3	Rough and granular	-	*Acaulospora*
68	AMF D16(e)	124.8	Yellow	4.7	Rough and granular	-	*A.scrobiculata*
69	AMF E1	152.7	Yellow	3.2	Smooth	30.2	*A.morrowae*
70	AMF E3	151.5	Brown	5.6	Smooth	28.9	*A.lacunosa*
71	AMF E4	152.3	Yellow	4.5	Smooth	-	*G.lacteum*
72	AMF E5	98.8	Yellow	4.7	Smooth	-	*G.mosseae*
73	AMF E7(a)	98.2	Brown	3.6	Rough	-	*A.scrobiculata*
74	AMF E8	103.8	Brown	4.5	Rough	-	*G.fasciculatum*
75	AMF E10	140.6	Yellow	6.3	Smooth	46,3	*G.mosseae*
76	AMF E11	105.3	Yellow	3.2	Smooth	67.8	*G.fsciculatum*
77	AMF E12	121.8	Yellow	5.7	Smooth	-	*G.etunicatum*
78	AMF E13	196.3	Brown	8.4	Smooth	-	*G.versiformae*
79	AMF E15	86.8	Yellow	3.4	Rough	15.2	*G.multicaulis*
80	AMF E16	83.5	Yellow	3.2	Rough	-	*G.mosseae*
81	AMF B1	132.5	Brown	5.3	Smooth	-	*G.fasciculatum*
82	AMF B2	112.3	Yellow	4.8	Smooth	-	*G.mosseae*
83	AMF B3	82.3	Yellow	3.7	Rough	-	*G.occultum*
84	AMF B4	153.3	Yellow	9.6	Smooth	45.7	*Entrophospora singulae*
85	AMF B5	158.6	Yellow	5.7	Smooth	-	*Acaulospora*
86	AMF B7	152.6	Yellow	9.3	Smooth	-	*G.radiatum*
87	AMF B9	99.6	Brown	3.2	Smooth	-	*G.macrocarpum*
88	AMF B10	185.2	Yellow	6.3	Rough	-	*A.lacunosa*
89	AMF B11	160.4	Yellow	9.7	Smooth	10	*Acaulospora*
90	AMF B12	84.5	Yellow	8.9	Smooth	-	*G.multicaulis*
91	AMF B17	174.7	Yellow	8.9	Smooth	-	*A.morrowe*

92	AMF B18	213.8	Black	7.2	Smooth	-	*G.farmosanum*
93	AMF B22	173.3	Brown	5.3	Rough	-	*Acaulospora*
94	AMF B22(a)	208.6	Brown	5.6	Rough	-	*Acaulospora*
95	AMF B23	86.2	Black	8.2	Smooth	-	*G.ambispora*
96	AMF B24	109.2	Yellow	5.7	Smooth	-	*Acaulospora*
97	AMF B26	92.5	Yellow	-	Rough	-	*Acaulospora*
98	AMF B27	75.6	Yellow	4.5	Rough	-	*A.sporocarpum*
99	AMF B29	70.9	Brown	3.8	Smooth	23.9	*G.mosseae*
100	AMF B32	93.5	Yellow	4.4	Smooth	-	*A.dilatata*

Molecular characterization of efficient native AMF by amplification of ITS region

Molecular characterization of efficient native AMF by amplification of its region of 18s rDNA subunit

As many as five morphotypes were chosen based on the abundance in the all five altered conditions compared to other morphotypes. These five AM fungal morphotypes were genetically characterized by PCR amplification of internal transcribed species of the ribosomal DNA (ITS18S rDNA). The obtained morphotype was confirmed as *Glomus mosseae* by amplifying with the *Glomus* specific primers GloITS-I and GloITS-II (Jansa *et al*., 2002 and Jones *et al.,* 2012).

In conclusion, thirty nine species of AM fungi were identified from five altered condition. Twenty species were recorded under elevated CO_2 plus temperature condition; fourteen species were recorded in reference plot; thirteen species were recorded under elevated CO_2 condition; eleven species were recorded under elevated temperature condition and lowest AMF species were recorded under ambient condition. In which, *Glomus fasciculatum* spores were most abundant in elevated CO_2 plus temperature condition as well as in elevated CO_2 condition, while *Glomus mossae* recorded maximum spore abundance in elevated temperature, ambient and in open reference ring.

The highest Shannon diversity index was recorded under elevated CO_2 plus temperature condition based on spore count. However, highest species richness was recorded in the soil received elevated CO_2 plus temperature condition.

The results of the morphological characterization of the native isolates under five altered conditions have clearly revealed that *Glomus mossae* was the most predominant species in all the five conditions based on their occurrence and abundance. Further, the native *Glomus mossae* isolates were genetically authenticated by the amplification of 375 bp specific DNA band in the ITS region of AM fungi. Thus, the present investigation will bring out an efficient climate resilient AMF culture collection to sustain the soil health under the anticipatory climate change.

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References

Anita, A., Peter, B.R. and Nancy, C.J., 2011, Seven years of carbon dioxide enrichment, nitrogen fertilization and plant diversity influence arbuscular mycorrhizal fungi in a grassland ecosystem. *J. New Phytolo.,* 192: 200-214.

Auge, R.M. 2001, Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza.,* 11: 3–42.

Gavito, M. E., Curtis, P. S., Mikkelsen, T. N. and Jakobsen, I., 2000, Atmospheric CO_2 and mycorrhiza effects on biomass allocation and nutrient uptake of nodulated pea (*Pisum sativum* L.) plants. *J. Exp. Bot*., 51: 1931–1938.

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

IPCC, 2007, Climate change 2007, The

physical science basis, contribution of working group-I to the fourth assessment report of the intergovernmental panel on climate change. Cambridge: Cambridge University Press.

Jansa, J., Mozafar, A., Banke, S., Mcdonald, B. A. and Frossard, E., 2002, Intra and inter sporal diversity of ITS rDNA sequences in *Glomus intraradices* assessed by cloning and sequencing, and by SSCP analysis. *Mycol. Res*., 106: 670–681.

Jones, N. P., Krishnaraj, P., Kulkarni, J. H., Chettri, P., Patil, A., Alagawadi, A., Vasu, D., 2013, Oral presentation at 7th international conference on mycorrhiza, “Mycorrhiza for all: An under- earth revolution” The energy and resources institute, New Delhi, India, 6-11.

Margalef, R., 1958, Information theory in ecology. *Gen. Systems*, 3: 36-71.

Maria, O., Garcia, Tatevik, O., Mary, G., Kathleen, K. and Treseder, 2008, Mycorrhizal dynamics under elevated CO_2 and nitrogen fertilization in a warm temperate forest. *J. Plant Soil,* 303: 301–310.

Shannon, C. E. and Weaver, W., 1949, The mathematical theory of communication. University of Illinois press. Urabana.

Stephane, C., Marcel, G. A., Heijden, V. D. and Angela, S., 2010, Climate change ejects on beneficial plant microorganism interactions. *J. Microbiol Ecol.,* 73:197-214.

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