Identification of Arbuscular mycorrhizal multiplicity in the saline-sodic soils

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Abstract: This study focused on the Arbuscular mycorrhizal (AM) fungal diversity in the saline-sodic soils based on native spore density and most probable number (MPN) assay. Identification through spore morphology showed existence of five genera in the various crop rhizospheres. The physico-chemical analysis of the native soils revealed that they were saline-sodic with pH ranging from (8.7±0.5) to (9.5±0.6) and habituated five different genera of AM fungi including Glomus, Scutellospora, Acaulospora, Sclerocystis and Gigaspora. Each location revealed presence of varied species of AM fungus namely Acaulospora and Glomus in rhizosphere of maize; Scutellospora and Glomus in tulsi; four isolates of Glomus in onion; Glomus and Sclerocystis in guava; three isolates of Glomus in rice; Glomus in neem and Gigaspora and Glomus in bamboo. The molecular identification through nested PCR analysis showed amplification of 600 bp size in SSU rDNA gene in samples A and C (predominated by Acaulospora and Glomus mosseae respectively).

Keywords: AM fungal identification, saline-sodic soil, crop rhizosphere, spore density, nested PCR technology, glomalean fungus
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1 Introduction

Arbuscular mycorrhizal (AM) fungi are obligate symbionts known to colonize most cultivated plants, native grasses[1-3] herbaceous plants[4] and shrubs as well as forest tree species[5]. Approximately 83% of monocotyledons form mutualistic symbiosis[6,7] with mycorrhiza which is the most efficient mechanism for phosphorus (P) acquisition, especially under biotic/abiotic stress conditions. These AM fungi are the most widespread group[8,9] and nearly 150 species are classified under seven genera which are able to form arbuscules *viz. Acaulospora, Entrophosphora, Gigaspora, Glomus, Sclerocystis and Scutellospora*. They exist in diversified environments, over a wide range of soil pH from 4.0 - 9.0[10], soil phosphate levels[11,12], salinity[13] and show non-host specificity by colonization in roots of plants grown in an Evergreen Montane forest[14], trees of Indian deserts[15], pteridophytes[16] and several medicinal plants[17]. Variation in the populations of these fungi and their symbiosis with plant roots is related to soil properties and host plants[18]. Relatively large populations of these fungi have been reported in some saline soils[19,20] and in addition, species and isolates of AM fungi differ in their tolerance to adverse physical and chemical conditions in soil[19]. There are, however, marked differences considering distribution and abundance among species and strains of AM fungi in response to soil properties. The last decades brought significant advancements to the understanding and appreciation of the kingdom fungi especially the

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Glomeromycotans to ecologists because of its potential influence on ecosystem processes, its role in determining plant diversity in natural communities and the ability of the fungi to induce a wide variety of growth responses in coexisting plant species.

Identification of AM fungi in soils has been carried out mainly by analysis of spores through morphological characteristics or more recently, by PCR methods (Polymerised Chain Reaction)[21]. Several PCR based methods have been developed over recent years, to obtain sufficient quantities of DNA, as these organisms are non-culturable and thus only a small quantity of DNA can be isolated from spores and infected roots. Studies on molecular diversity of AM fungi and patterns of host association over time period of three consecutive years in a tropical forest resulted in a total of 30 AM fungal types[22]. Advances in molecular biology and an increased understanding of the phylogeny of AM fungi, have paved the way for molecular methods of fungal identification[23,8] largely using PCR technology.

AM fungus *Glomus intraradices* from the roots of lettuce, zinnia, leek, pepper and endive plants was detected by using a PCR primer pair[24]. The discriminating probes, derived from the variability of the 5' end of the large ribosomal gene subunit of *Glomales*, have been obtained and a nested PCR technique[25] was developed to distinguish between different AM fungal species colonizing a mycorrhizal root system[26]. Group-specific primers were designed for five major phylogenetic lineages of AM fungi to amplify the highly variable ITS (internal transcribed spacer) and the AM fungi within colonized roots were identified using specific PCR primers[27]. This system was recently used to identify the fungal symbionts of mycoheterotrophic plants[28]. Difficulties in identification, problems of taxonomic classification and a lack of basic information on the life histories of AM fungi hinder studies of the ecological significance of diversity of AM fungi. Nucleic acid based techniques have the potential to fill this gap in our knowledge by offering better means of identification and the opportunity to study the links between the genetic, functional and morphological diversity of AM fungi. Generally the sodic soils are those which hold pH value at alkaline range up to 9.5 and since surveying for the native AM fungal species in such a habitat would result in strains that survive salt stress. The study was undertaken to assess the diversity of AM fungi in sodic soils of selected plants by morphological examination of AM fungal spores.

2 Materials and methods

2.1 Sampling

Sampling occurred at Anbil Dharmalingam Agriculture College and Research Institute (ADAC & RI) campus located at an altitude of 85 meters above MSL (Mean Sea Level), 10°45′N latitude and 78°36′E longitude at Trichy district, Tamil Nadu state, India. Seven different crop rhizosphere *viz.* maize, tulsi, onion, guava, rice, neem and bamboo were marked as sampling sites and top soil (0-20 cm) were collected and homogenized. One kg of each soil sample was packed separately in polyethylene bags and stored. The samples were taken for estimation of their physico-chemical properties *viz.* pH (ELICO pH meter) & electrical conductivity (EC) (CM 180 Elico conductivity Bridge), Exchangeable Sodium Percentage (ESP), available N (nitrogen) (Alkaline potassium permanganate method), available 'P' (phosphorus) ($NaHCO_3$ extract-Colorimetric method) and available K (potassium) (Neutral normal ammonium acetate extract-Flame photometer) using standard methodologies.

2.2 Physical analysis

To assess the AM population, soil samples were assayed for the infective propagule (IP) count through Most Probable Number (MPN) technique[29] as described below. All seven rhizosphere soil samples were diluted serially using sterile sand. The diluted samples were placed in black polyethylene tubes each with five replications. Surface sterilized maize seeds (COH1 MI5 hybrid) were sown in each tube and the plants were grown for a period of six weeks *in vitro*. After six weeks, the plants were uprooted and root samples were collected from each replication for assessment of total root colonization by adopting the using magnified intersection method as described[30]. The number of positive tubes (with AM colonization) for three subsequent dilutions was

observed for the MPN index value by referring to the MPN table. MPN index value was multiplied with middle dilution factor to calculate the IP via:

$$Number\ of\ IP\ g^{-1} = \frac{MPN\ index\ value \times Middle\ dilution\ factor}{Dry\ weight\ of\ the\ soiltaken} \times 100 \quad (1)$$

After the assessment of IP through the MPN technique, the rhizosphere soils (sample A, B, C, D, E, F, G) were maintained as trap cultures[31] for multiplication of spores using maize as host crop. AM fungal spores isolated using the method of wet sieving and decantation technique[32] from the trap culture soil were used for identification through morphological and molecular studies. A 100 g AM inoculum (the trap cultured soil) was taken in a 1 L beaker, suspended in 1 L of water, stirred well and heavier particles to settle for a few seconds (ca. 20 s). The suspension was passed through a coarse sieve (2 mm) to remove large pieces of debris. The process was repeated a second time to ensure proper homogenization. The suspension was collected and passed through a series of sieves (180, 106 and 45 μm) arranged in the descending order of their mesh size and the fungal propagules were collected from each sieve separately. This process was repeated 4 to 5 times until the suspension appeared clear. The propagules from all the three sieves were pooled and finally observed under stereoscopic zoom microscope for the presence of spores. Young spores were collected using a capillary tube into a sterilized vial containing sterile distilled water.

Spores with similar morphological features were isolated from each soil sample to calculate the spore density. After extraction of spores from soil, intact spores were picked up using a wet needle and mounted in polyvinyl alcohol lactoglycerol (PVLG) and Melzer's reagent on a glass slide for identification as described[33]. The spores were differentiated based on spore colour, size, hyphal attachment, hyphal thickness, wall characteristics and other features as described[34]. The macro-characteristics *viz.* sporocarp, spore, subtending hypha were used to characterize the visual differences in the gross descriptive morphology of a species[35]. To have a more precise identification of AM fungi, the micro-characteristics *viz.* spore wall layer, texture and colour were studied[36] to differentiate among certain AM fungi using visual differences in morphology of fungal hyphae and vesicles within the roots. The mounted spores were identified based on their morphological characteristics and designated.

2.3 PCR analysis

Molecular identification of AM fungus was carried out by polymerase chain reaction (PCR) method. The DNA extraction was done from the AM fungal spores isolated from the trap culture as well as host plant root[37]. Total genomic DNA extraction was carried out with AM fungal spores of the various morphotypes isolated from the seven soil samples. Approximately 20 to 40 spores each were placed in microcentrifuge tubes containing 40 μL milli Q–water and subjected to alternate freezing (liquid nitrogen) and thawing (room temperature) ca. five times and crushed with a miniature pestle. A 13 μL aliquot of 100 mM Tris HCl (pH 8.0) was added to crushed spores. The suspension was vortexed vigorously and heated to 95℃ for 5 min. The samples were placed in ice for 5 min and then centrifuged at 10 000 r/min for 2 min. The supernatant of each sample was frozen at -20℃ and were used as template for PCR. The DNA extraction was also done from the roots of maize crop grown under trap culture. Roots were grounded using liquid nitrogen and of the powdered sample 0.5 g was weighed and taken for DNA extraction, carried out using DN easy plant kit (QUIAGEN) and the extract obtained through the kit contained mixture of plant as well as fungal DNA which was taken for PCR amplification.

The total DNA extracted from the spores as well as from the roots, was taken for PCR amplification. The first set of PCR was done using the universal primer NS5_F (5' - AAC TTA AAG GAA TTG ACG GAA G - 3')[38] and the fungal primer ITS4_R (5' - TCC TCC GCT TAT TGA TAT GC - 3') [26]. Nested PCR was carried out using first PCR product (diluted 1, 50) as a template using the universal primer NS21 (5' – AATATACGCTA TTGGAGC TGG; - 3') and the AM taxon specific primer VANS1 (5'-GTCTAG TATAATCGTTATACAGG -3')[39] with slight modification in the annealing temperature of 61℃ for 1 min.

2.4 Statistical analysis

The data were subjected to statistical analysis by variance (p=0.05) with mean separation by Least Significant Difference (LSD)[40]. The analysis for microbial population count was based on the log and arcsine transformed values. A simple correlation analysis (p = 0.05) was carried out between soil physico-chemical properties and spore density of AM fungi in native soil.

3 Results

3.1 Physico-chemical characteristics

The physico-chemical properties of the soil samples collected from seven different locations of ADAC & RI campus, Trichy exhibited that all the soils had similar sodicity, however differed in pH under alkaline range varying from (8.7±0.5) to (9.5±0.5) (Table 1a). The EC level was slightly varying between the locations ranging from a minimum of (0.07±0.01) to a maximum of (0.25±0.02). The ESP of soil ranged from (15.5±0.8) to (16.8±0.9) where the highest was observed in the rhizosphere soil of rice. The rhizosphere soil of tulsi ranked the highest in available N while available P was the highest in the rhizosphere of maize and available K being the highest in rhizosphere of guava (280 kg ħa^{-1}).

Table 1 Physico - chemical properties of sodic soils collected from ADAC & RI, Trichy

Sample	Rhizosphere	pH value	EC/dsM^{-1}	ESP/%	N content /kg ħa^{-1}	P content /kg ħa^{-1}	K content /kg ħa^{-1}	AM spore density /100 g^{-1} soil
A	Maize	8.7±0.5	0.10±0.01	16.5±0.9	156.84±1.8	27±1.7	210±12.1	110±7.0
B	Tulsi	8.6±0.5	0.25±0.02	16.5±0.9	219.52±2.5	10±0.6	196±2.2	90±5.7
C	Onion	8.5±0.5	0.23±0.01	16.0±0.9	188.16±2.1	20±1.2	270±2.6	85±5.4
D	Guava	8.8±0.5	0.18±0.01	15.5±0.9	125.44±1.4	15±0.9	280±2.7	140±8.8
E	Rice	9.5±0.5	0.21±0.01	16.8±0.9	145.53±1.6	22±1.4	250±2.4	70±4.4
F	Neem	8.7±0.5	0.07±0.01	15.7±0.9	94.08±1.0	12±0.7	150±1.7	80±5.0
G	Bamboo	8.8±0.5	0.10±0.01	15.5±0.9	93.57±1.0	13±0.7	145±8.3	120±7.6

Note: Values are mean (± standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by Least Square Means test (p=0.05).

3.2 AM fungal spore density and IP count

The spore density varied in each rhizosphere location with the rhizosphere soil of guava having the highest spore density while rhizosphere of bamboo and maize were next highest (Table 1a). The correlation analysis of the soil physico-chemical properties with AM fungal spore density showed negative for soil Phosphorus, pH and EC status (Table 1b). Among the IP assessed, the RLH (Root Length Hyphae) was maximum in sample B and sample C showing (37.31±2.37)% and (37.11± 2.36)% respectively. The RLV (Root Length Vesicles) was highest in sample E (24.38 ±1.55)% while RLA (Root Length Arbuscules) was highest in sample B showing (1.06±0.07)%. Also the RLC (Root Length Coils) was higher in sample B with (9.08±0.58)%. The RLTC (Total Root Length Colonization) was assessed based on the totality of the fungal structures present which showed sample C ranked highest (54.32±3.45)% (Table 2) (Figure 1). In total, the IP were recorded highest in the sample C (0.540 × 10^2 100 g^{-1} soil) followed by sample A (0.470 × 10^2 100 g^{-1} soil) while the least count was observed in sample G (0.120 × 10^2 100 g^{-1} soil).

Table 2 Correlation coefficients between soil physico-chemical properties and AM spore density

Physico-chemical characteristic	Nutrient content/kg ħa^{-1}			Spore density
	N	P	K	
pH	-0.257	0.618	0.244	-0.276
EC	0.774	0.254	0.657	-0.254
Spore density	-0.297	-0.092	0.138	1

Note: Values determined by Least Square Means test (p=0.05).

3.3 Morphological diversity

The AM genera and species were identified based on their morphology revealing five genera *viz. Glomus*, *Scutellospora*, *Acaulospora*, *Sclerocystis* and *Gigaspora*. Among the eighteen isolates observed, thirteen were classified under the genus *Glomus*, two under *Scutellospora*, one under *Acaulospora*, one under *Sclerocystis* and one under *Gigaspora*.

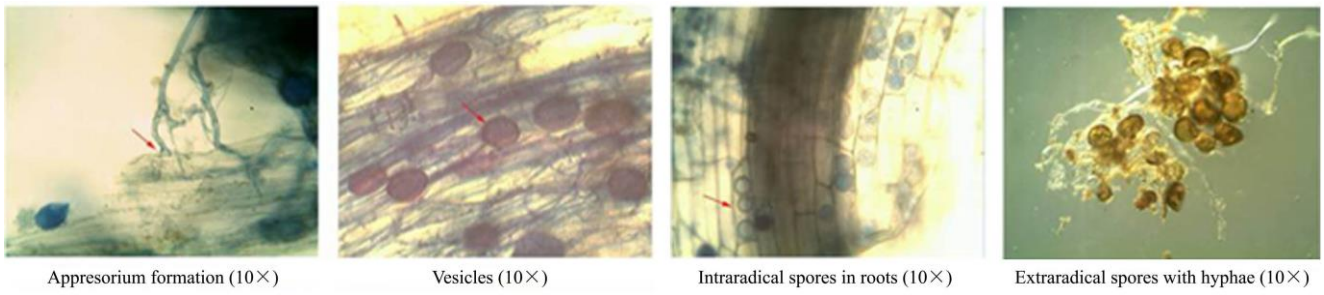


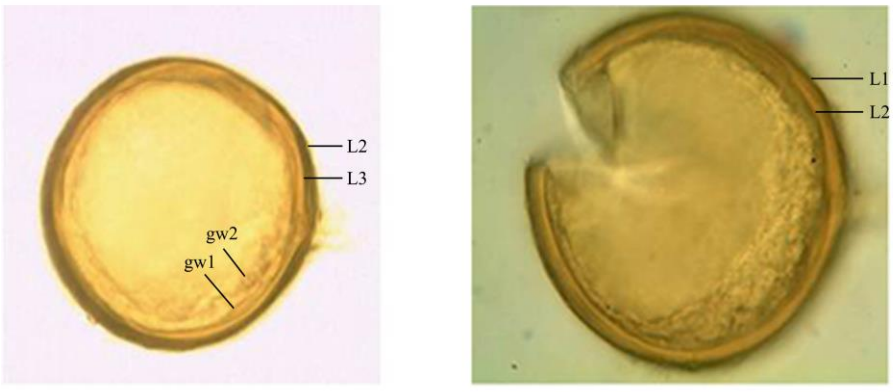
Figure 1 Infective propagules of AM fungl observedin roots of maize grown in sodic soils under MPN technique

In each location of soil sampling, more than one species of AM fungi were isolated (Table 3). In the rhizosphere soil of maize (sample A, Figure 2), one isolate of *Acaulospora* sp. and one *Glomus* (*G. etunicatum*) were found. In rhizosphere soil of tulsi (sample B, Figure 3), two isolates of *Scutellospora* (*S. calospora*; *S. heterogama*) and two isolates of *Glomus* (*G. intraradices*, *G. etunicatum*) were observed and in rhizosphere soil of onion (sample C, Figure 4), all the isolates belonged to the genus *Glomus* (*G. fasciculatum*, *G. clarum*, *G. mosseae* and *G. claroideum*). Two different genus were observed in rhizosphere soil of guava (sample D, Figure 5), one isolate of *Glomus* sp. and one *Sclerocystis* sp. Rhizosphere soil of rice (sample E, Figure 6) showed three isolates *Glomus* (*G. geosporum*, *G. intraradices*, *G. albidum*) and rhizosphere soil of neem (sample F, Figure 7) had only one isolate of *Glomus* (*G. aggregatum*) while one isolate of *Gigaspora* (*Gigaspora* sp.) and one isolate of *Glomus* (*G. viscosum*) were identified in the rhizosphere of bamboo (sample G, Figure 8). Apart from the diversity in each rhizosphere the percentage of occurrence of each species marked their predominance.

Table 3 Enumeration of infective propagules (IP) and total root colonization percentage in maize roots raised under MPN technique

Sample	Rhizosphere	Infective propagules /$\times10^2$ 100 g^{-1} soil	Total root colonization (units 50 observation points $^{-1}$)				
			RLH/%	RLV/%	RLA/%	RLC/%	RLTC/%
A	Maize	0.470±0.005^{b}	35.55±2.26^{b}	12.21±0.78^{d}	0.17±0.01^{f}	2.42±0.15^{c}	50.35±3.20^{b}
B	Tulsi	0.330±0.004^{c}	37.31±2.37^{a}	5.63±0.36^{e}	1.06±0.07^{a}	9.08±0.58^{a}	53.08±3.37^{ab}
C	Onion	0.540±0.006^{a}	37.11±2.36^{a}	13.39±0.85^{c}	0.27±0.02^{e}	3.55±0.23^{b}	54.32±3.45^{a}
D	Guava	0.200±0.002^{d}	22.09±1.40^{e}	15.75±1.00^{bc}	0.33±0.02^{d}	3.55±0.23^{b}	41.72±2.65^{d}
E	Rice	0.140±0.002^{f}	25.82±1.64^{d}	24.38±1.55^{a}	0.70±0.04^{b}	-	50.90±3.23^{b}
F	Neem	0.170±0.002^{e}	27.95±1.78^{c}	16.55±1.05^{b}	0.34±0.02^{d}	-	44.84±2.85^{c}
G	Bamboo	0.120±0.001^{g}	20.11±1.28^{f}	2.27±0.14^{f}	0.47±0.03^{c}	-	22.85±1.45^{e}

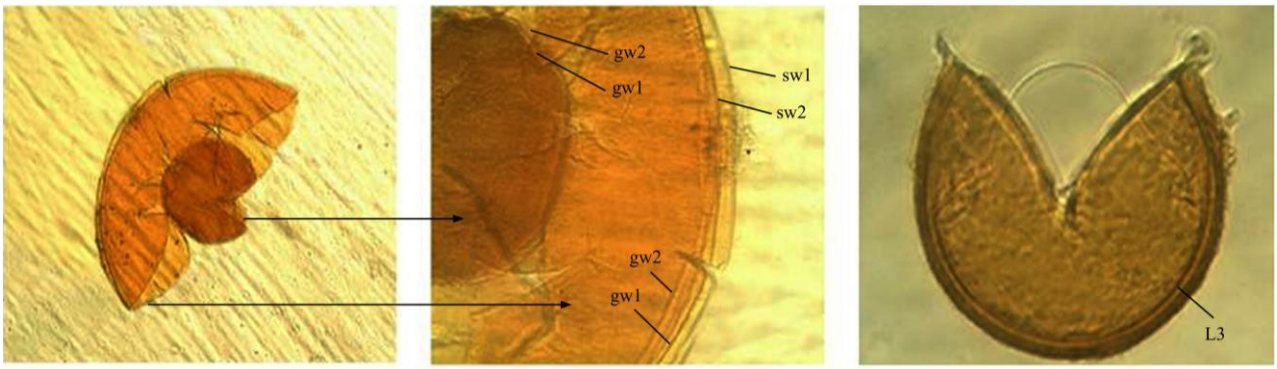
Note: RLH - Root Length Hyphae; RLV - Root Length Vesicles; RLA - Root Length Arbuscules; RLC - Root Length Coils; RLTC - Root Length Total Colonization. Values are mean (± standard error) (n=3) and values followed by the same letter in each column are not significantly different from each other as determined by Least Square Means test (p=0.05).



Acaulospora sp. *Glomus etunicatum*

L1: Laminae 1; L2: Laminae 2; L3: Laminae 3; gw1: germinal wall alyer 1; gw2: germinal wall alyer 2

Figure 2 AM fungal diversity in rhizosphere of maize in sodic soil (40×)



Scutellospora calospora (10×) *Glomus intraradices* (40×)

Figure 3 AM fungal diversity in rhizosphere of Tulsi in sodic soil

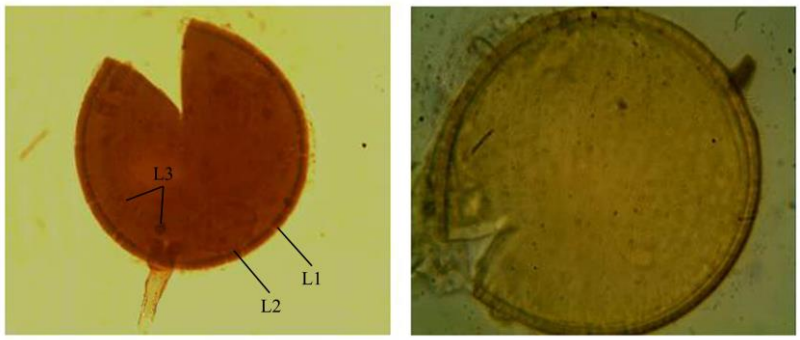
Glomus fasciculatum *Glomus clarum* *Glomus claroideum* *Glomus mosseae*

Gt: germ tube; L1: Laminae 1; L2: Laminae 2; L3: Laminae 3

Figure 4 AM fungal diversity in rhizosphere of onion in sodic soil (10×)

Glomus sp. (40×)

Figure 5 AM fungal diversity in rhizosphere of guava in sodic soil

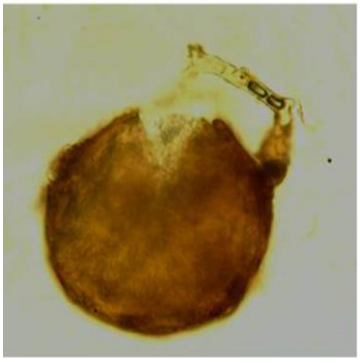


Glomus geosporum (10×) *Glomus albidum* (40×)

Figure 6 AM fungal diversity in rhizosphere of rice in sodic soil

G. aggregatum

Figure 7 AM fungal diversity in rhizosphere of neem in sodic soil



Gigaspora sp.

Figure 8 AM fungal diversity in rhizosphere of bamboo in sodic soil (10×)

Table 4 Morphological identification of AM fungal isolates in sodic soils

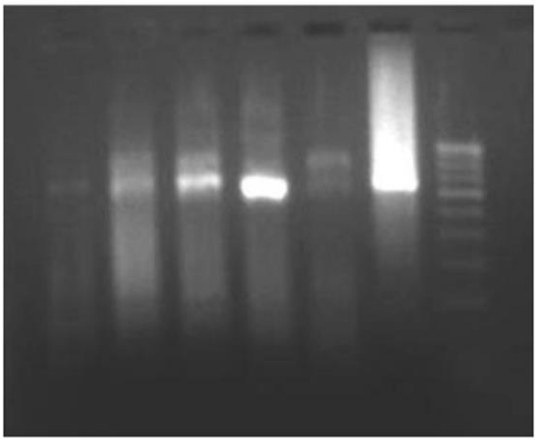
Sample	Rhizosphere	Tentative identification	Per cent occurrence	Description	Figure No.
A.	Maize	**Acaulospora* sp.	80	Globose to Subglobose spores, pale yellowish in colour with three spore wall layers (L1, L2, L3) namely outer hyaline layer (L1) membranous light yellow coloured layers (L2 and L 3). Matured spores showed two germinal wall layers (gw1, gw2) and sometimes with only one germinal wall layer.	Figure 2
		Glomus etunicatum	20	Light brown coloured spores, globose with spore wall appearing dark brown in colour and the spore surface appearing golden yellow. Spores had two wall layers namely outer very thick (L1) and inner thin wall (L2) and with septum in their hyphae was a typical character of this species.	
B.	Tulsi	**Scutellospora calospora*	40	Globose shaped milky white spores. Spore wall composed of two tightly adherent layers. First layer (SW1) was permanent smooth and hyaline while second (SW2) was laminate, yellow coloured. Also the germinal wall layers consisted of two flexible hyaline layers (GW1, GW 2). GW1 had two layers, tightly adherent to each other and in GW2 layer 1 was flexible, hyaline stained dull red in melzer's reagent while layer 2 appeared plastic, hyaline and stained thick in melzer's reagent	Figure 3
		Scutellospora heterogama	10	Subglobose to oblong shaped spores, dark brown in colour with mutilayered walls and an inner wall of coriaceous (C) type possessing a bulbous hyphal base. These spores had germinal walls which were bilayered and flexible (synthesized consecutively after the spore wall has completed differentiation). Each had two layers (L1 and L2) that were tightly adherent and appeared as one layer when mounted in PVLG and therefore could not be differentiated layer wise.	
		Glomus intraradices	15	Globose or subglobose, brown coloured spores with outer dark margin and inner light margin showed one clear wall layer (L3). Spore Surface pitted found with small notches.	
		Glomus etunicatum	35	Brown coloured spores had two wall layers of evanescent (E) type. Hyphae consisted of thin adherent laminae, light brown in colour.	
C.	Onion	*Glomus fasciculatum*	15	Globose, ellipsoidal and some irregular spores were found with interwoven hyphae. Spore walls had double layers coloured light yellow. The spores appeared yellowish having outer wall entire and inner wall irregular.	Figure 4
		Glomus clarum	20	Globose spores with subtending hyphae, cylindrical to flared, constricted. These spores showed three layers (L1, L2 and L3), with L1 (mucilagenous), the only layer present in juvenile subtending hyphae, thinning to invisibility within 5 μm of the spore surface. A germ tube emerged from the lumen of the subtending hypha and originated either at the septum or at a break in branch hyphae. It appeard to arise from the innermost sublayer of L3.	
		**Glomus mosseae*	50	Golden yellow to brown coloured spores, globose, with funnel shaped hyphal base divided from subtending hyphae by a curved septum marked the characteristic feature of this species	
		Glomus claroideum	15	Cream to light yellow coloured spores, globose to subglobose in shape. The spore walls were hyaline mucilaginous and difficult to distinguish as both layers were so tightly adherent and appeared granular. There were approximately four spore wall layers (L1, L2, L3 and L4) where, only the first two hyaline layers were continuous with the juvenile wall of the subtending hypha. L3 developed next in both the subtending hypha as well as spore, gradually thickened to eventually make up most of the spore wall thickness. L4, originated as part of the subtending hyphal wall, but only in the region of the spore base. L3 consisted of thin and tightly adherent pale yellow with laminae.	
D.	Guava	*Glomus* sp.	15	Single spores with multilayered wall. Colour of the spore walls ranged from hyaline to yellow	Figure 5
		**Sclerocystis* sp.	85	Sporocarps globose, subglobose, brown to blackish brown, without peridium, had one to four monohyphal stalks; chlamydospores brown to dark brown, developed asynchronously. In the same sporocarp, club-shaped vesicles (young spores) were often observed intermingled with mature spores.	
E.	Rice	**Glomus geosporum*	65	Reddish brown smooth walled spores, with lengthy tube like subtending hyphae, which was hyaline was the characteristic feature of *G. geosporum*. Spores showed three wall layers (L1, L2, L3) which were tightly adherent, laminated and membraneous. L1, a hyaline sloughing granular layer; L2, a rigid layer consisted of adherent sublayers appeared orange-brown in color, L3, a semi-rigid usually resolved by slightly darker color (yellow to orange-brown), continued growth into the lumen of the subtending hypha to form an unbroken recurved septum. This layer appeared to have short stubby warts on the inner surface. A germ tube emerged from the lumen of the subtending hypha, originated from the recurved septum.	Figure 6
		Glomus intraradices.	30	Single spores borne on a swollen hyphae appeared brown in colour.	
		Glomus albidum	5	Single spores borne on a swollen hyphae appeared dark brown in colour.	
F.	Neem	**Glomus aggregatum*	100	Light brown coloured small sized spores were found clustered with hyphal connections, occurred mostly in root bits in coarse fraction of the sievings.	Figure 7
G.	Bamboo	**Gigaspora* sp.	55	Dark brown spores, subglobose in shape, composed of 2 wall-layers. These spores were observed with bulbous stout hyphae with tapering end, the characteristic feature of this genus.	Figure 8
		Glomus viscosum	45	Pale white coloured spores with lengthy and clustered hyphae, appeared brown in colour under PVLG + Melzer's reagent. Spores always adhered to soil particles.	

Note: * Predominant spore type of AM fungi in the respective rhizosphere.

In rhizosphere soil of maize, *Acaulospora* sp. was predominant (80%) than *Glomus* and in the rhizosphere soil of tulsi, *Scutellospora calospora* was more (40%) than *Glomus*. In the rhizosphere soil of onion, *G. mosseae* occurred up to 50% than the other species of *Glomus* while *Sclerocystis* sp. (85%) was predominant in rhizosphere soil of guava. Rhizosphere of rice showed 65 % of *Glomus geosporum* while rhizosphere of bamboo had predominance of *Gigaspora* sp. (55%) and that of neem with only *Glomus aggregatum* (Table 3). In the present study, survey in sodic soils showed AM fungal diversity of 18 isolates belonging to five genera (*Glomus, Gigaspora, Scutellospora, Acaulospora* and *Sclerocystis*) under the pH of 8.5 to 9.5 where, *Acaulospora* was predominant at pH 8.7, *Scutellospora* at pH 8.6 and *Glomus* at pH 8.5, 9.5 and 8.7.

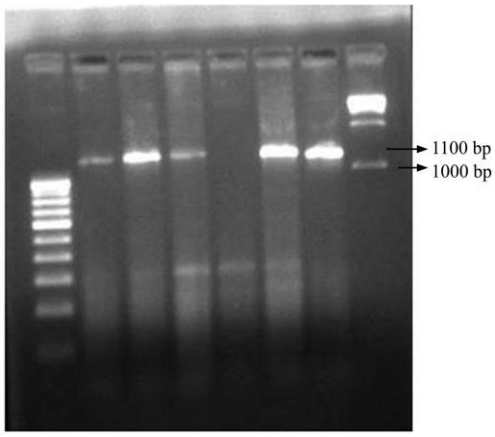
3.4 Molecular diversity

The seven different isolates of AM fungi identified through morphology were used for confirmation through molecular study. Results showed PCR amplification of five isolates *viz. Acaulospora* sp., *Scutellospora* sp., *Glomus mosseae*, *Sclerocystis* sp. and *Glomus geosporum* with uniform bands at 600 bp (Figure 9). First PCR analysis resulted in amplifications of 1100 bp size (Figure 10a) in five samples (A, B, C, E and F) while the results of nested PCR showed amplification of 600 bp size (Figure 10b) in the samples A and C (predominated by *Acaulospora* and *Glomus mosseae* respectively). In present study, two AM isolates A and C showed amplification of 600 bp in SSU rDNA gene.



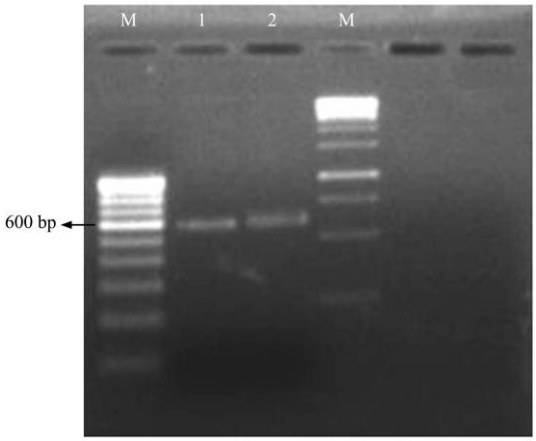
Lane: 1 - *Acaulospora* sp. 2 - *Scutellospora* sp. 3 - *Glomus mosseae* 4 - *Sclerocystic* sp. 5 - *Glomus geosporum* 6 - Positive control M – Marker

Figure 9 PCR detection of AM fungal spores



Lane: 1 - Sample A 2 - Sample B 3 - Sample C 4 - Sample A 5 - Sample E 6 - Sample F

a. PCR detection of AM fungus in mycorrhizal roots under trap culture



Lane: 1 - Sample A 2 - Sample C M – Marker

b. Nested PCR detection of AM fungus in mycorrhizal roots under trap culture

Figure 10 Detection of AM fungus by PCR and nested PCR

4 Discussion

4.1 Physico-chemical characteristics of sodic soil samples

The physico-chemical characteristics of the soils indicated variations with respect to soil pH, EC and fertility status. The pH values of all the soil samples were ranging from neutral to alkaline (pH 8.5 to 9.5) with EC < 1. Available nutrient status showed low nitrogen with medium range of phosphorus and potassium content[41]. Nutrient deficiency particularly with low level of organic carbon and phosphorus with moderate to high level of potassium has also been reported in the coastal saline soils[42]. The differences in spore density were observed in the rhizosphere of each crop and such variation in spore density in different soils may be due to increase in root colonization which is directly related with

the germination the fungal spores[43] in the presence of living host roots, infection by soil fauna or disturbance by fungal or other parasites.

4.2 Correlation study

The soil physico-chemical properties (soil pH, EC and available P) correlated with soil spore density resulted with negative values which showed that spore density is affected when there are changes in the soil properties. The spore density ranged the highest of 140 numbers at the lowest pH 8.8 and lowest of 70 at the highest pH 9.5. This is in accordance with few studies[44,45] which documented that root colonization and AM fungal sporulation can decrease with increasing levels of extractable soil P (Troech and Loynachan 2003). A study reported that a strong negative correlation (r = -0.865; p = 0.05) existed between IP of AM fungi and soil EC in the coastal lands of Mangalore[46]. The AM fungal diversity study in alkaline soils[47] registered higher density of 420 - 820 spores at pH of 7.8 - 7.4 and very less of 36 - 180 spores at pH of 8.8 - 8.2. Such a negative correlation was due to the better surveillance of the spores only at neutral to less alkaline soils than in alkaline or saline soils. Spore multiplication increases under low-phosphate conditions when there is starvation for the nutrient due to which the spore density is always high in soil with low 'P'[48] and these suggested that increase in P content of soil lead to hyphal spread and colonization which facilitate mobilization of the nutrients.

4.3 Infective propagules

IP count estimated through magnified intersection method showed *Glomus geosporum* to have maximum colonization with abundance of arbuscules and vesicles in the colonized maize roots. Formation of more arbuscules is an indication of active AM symbiosis are the important exchange sites in the root cortex for mineral elements[49]. Vesicle formation is a sign of the fungi speeding up their life cycles and the thick walled vesicles observed resemble spores, suggesting that they could function as propagules when isolated from roots[50] or support the regrowth of intercellular hyphae in appropriate conditions. MPN estimates of IP in AM fungi varied considerably amongst the seven soil samples ranging from 0.12 to 0.54 IP 100 g^{-1} soil. MPN number of propagules in soil depended on cropping management and soil type[51]. Mycorrhizal soil infectivity and AM fungal colonization upto 50% was examined in *Pterocarpus officinalis* in sodic soils and the impact of soil physico-chemical properties on variation of IP number was remarked[52]. Diversity and infectivity of AM fungi was studied in agricultural soils of the Sichuan Province of mainland China which showed that MPN values varied between 17 and 3 334 propagules 100 g^{-1} soil among the 50 field sites sampled[53]. Apart from estimating IP of AM fungi in various rhizosphere, studies to discover their diversity is further more interesting area wherein recent investigations focus.

4.4 AM fungal diversity

Several researchers have reported the wider occurrence of *Glomus* in several types of soils with many species of Glomalean fungi apparently adapted to diverse habitats[54]. Among the symbiotic association of various *Occimum* sp. with many genera of AM fungi, *Glomus aggregatum* and *Glomus fasciculatum* were predominant[55]. These findings are similar to the present study where, rhizosphere of tulsi was predominated by *Glomus* (*G. intraradices, G. etunicatum*) and *Scutellospora* (*S. calospora, S. heterogama*). Members of *Glomaceae* (*Glomus geosporum*) dominate upto 51.4% in the rhizosphere of *Phyla nodiflora*[42] and the present study also showed predominance of *Glomus.* Specificity in occurrence of AM fungi in certain rhizosphere have been linked with changes in soil fertility or cultural practices[56], environmental factors[57] and signaling by the '*myc'* factors (root exudates) in that particular soil and adaptation of this fungus to alkaline soils[58]. AM fungal species namely *Glomus mosseae, G. fasciculatum, Gigaspora margarita, G. gigantean* and *Acaulospora laevis* were identified in Jatropha planted soils that were alkaline in nature[59]. *Glomus mosseae* was found to be the most predominant (71.3%).

The identification of AM fungi at molecular level is concerned with the ITS region, which is the convenient target region due to its variability in length and in nucleotide content among different species. In the present study, PCR amplification of two isolates attained at 600 bp is in accordance with a finding[60] who reported

the presence of AM fungi in the colonized roots along with the finding that mycorrhizal root samples showed amplification at 372 to 660 bp but for the difference in species. Although the ITS region has proven useful for locating taxon specific primers for the *Glomus* sp., the results with *G. intraradices* strains suggest that there is too much intraspecific variation within the ITS regions of this broad group and often showed similarities with closely related *G. etunicatum* and *G. claroideum* strains[61]. *Glomus* sp. was identified at taxonomic level using primer pair VANS1 and NS21 with amplification of 550 bp region in SSU rDNA[62]. These reports confirmed that in this study, the samples A and C which showed amplification at 600 bp, could be *Acaulospora* sp. and *Glomus* sp. and this is in agreement with a study[63] which detected *Glomus* sp. in uninoculated soil and roots of turfgrass (*Agrostis palustris*) using the same set of primers.

5 Conclusions

The present diversity analysis brings out the facts of habitation of 15 species in a sodic soil site. Here, both morphological as well as molecular identification involved constraints in profound analysis of the spore wall layers and hyphal attachments in the former while its viability of the spores in the latter and because of which host roots were also tried in spite of using direct spores. Pyrosequencing of fungi in diverse environments, such as soil or roots, elevates the number of recovered taxa several fold. The use of genomic tools further will enable mycology to flourish in the near future with discovery of influential strains, making this a very exciting era to the mycologists.

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