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Isolation and characterization of arbuscular mycorrhiza from a newly developed L J farm at village Dumana, Viramgam, Gujarat

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Abstract

Mycorrhiza in general and arbuscular mycorrhiza in particular are the most important terrestrial fungi that evolved with the evolution of land and plants and became a part and parcel of the plant's growth and development. It is so important for plant nutrition that its density and diversity were studied worldwide in different habitats, niches and climates. In the present study, a spore density of 1.6 to 4.04 per g of soil was observed in a semi-arid region; agroclimatic zone V of Gujarat at Dumana L J Farm, Viramgam. Nine different species of endomycorrhizae were characterized; however, species of *Glomus* and *Acaulospora* were dominant taxa. Because there is little taxonomic diversity in such a large group of mycorrhizal fungi, it is crucial to analyze local populations that have adapted to different environments. Depending on the climate, each species may have a significant amount of genetic variation. The result shows the spore density in a developing plantation field, which was earlier mainly used for the cultivation of a local variety of cotton. Since there is no such report on the density and diversity of endomycorrhiza from this region, this primary study will serve as a baseline for the comparison of the spore density of future studies of the region/field in different plants and seasons.

Keywords

arbuscular mycorrhiza; glomeromycota; hyphal attachment; mycorrhiza; spore density; spore wall layer

Introduction

Mycorrhiza is a clear term for the naturally occurring symbiotic relationship between a plant and a fungus. The association is so unique that except ericoid, none of the endomycorrhizae is yet to be cultured axenically. This makes it very important from a microbiology, ecology and biotechnological point of view (1). Many years of research since its 1st report has proved its utility in soil, plant and ecosystem management for soil health, plant/crop health and environmental health (2). The taxonomy of arbuscular mycorrhiza is largely morphological. Spore morphology, spore production and spore wall structure were used to identify them (3-6). The fungi that generate arbuscular mycorrhiza were categorized in 1990 into one order, Glomerales (5), comprising 6 genera (*Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora*), 3 families (Gigasporaceae, Glomeraceae and Acaulosporaceae) and 1 phylum (Zygomycota). The phylum Glomeromycota was created as a result of the endomycorrhizae fungus and the morphological and molecular analyses that followed. Arbuscular mycorrhizal fungi (AMF) and vesicular arbuscular mycorrhiza (VAM) are 2 types of endomycorrhizae that are known to form in the phylum Glomeromycota; these endomycorrhizae have 3 classes, 5 orders, 15 families and 30 taxa that have been reported up until 2021 (7, 8). Still, the

spore formation is broadly classified as glomoid, gigasporoid, scutellosporoid, entrophosporoid, acaulosporoid, pacisporoid and biomorphic (5).

Although the traditional taxonomy is widely used in endomycorrhizal studies, there are limitations because non-sporulating species may be present and the relative abundance of a species spores may not be indicative of the amount and distribution of hyphae in the soil or the relative amount of fungus colonization of roots (9). Another issue with identifying the spores gathered from the field is spore wall degradation. Arbuscular mycorrhiza spore populations' quantitative and qualitative makeup is the outcome of intricate interactions between the fungus, the host plant and its environment. Since fungi sporulate in response to food limitation or other pressures, spore type and number indicate the relative importance of specific species within populations, but this cannot be correlated with their further infectivity (10). Because spores are available at various stages of development (11) and because the primary basis for assessing VAM and AMF species in field soils is spore wall features (12), it is not an easy task.

The present study examined the mycorrhizal diversity of non-agricultural sites at L J Farm, Dumana, Viramgam, Gujarat. The primary crop grown in the area, which borders L J Farm, is cotton. Understanding the endomycorrhizal spore types and density is crucial for the planting of different fruit orchards. To comprehend the significance of species composition and their association with abiotic conditions, the study examines the diversity of endomycorrhizal fungi at a non-agricultural site. Soil has been taken into account in the current effort to determine its importance. Arbuscular mycorrhizal (AM) fungi are very common and are known to be widely distributed. These fungi can be isolated from a wide range of natural settings. Regarding its dissemination in Gujarat, not much work has been done. New knowledge is being produced about AMF in dry environments that exhibit particular behavioral traits (13).

Materials and Methods

The current site of study comes under the arid zone, the northwest agro-climatic region of Gujarat, India. Dumana experiences 700 mm of yearly rainfall in a semi-arid climate. The medium-black soil at L J Farm has a sub-angular blocking structure and is plastic, hard, stiff and sticky. Its pH is 7.34, which is normal to saline and its organic carbon content is only 0.36%. It also has poor drainage. The soil has a rich potash content (387.82 kg/ha) and a medium phosphorus content (22.05 kg/ha). It has an EC of 0.40. The two main soil bottlenecks that prevent seed germination and a healthy plant population per unit area are water inundation and the subsequent crust development that occurs after drying. May and June see the highest temperatures (up to 45°C), while December and January have the lowest temperatures (up to 20°C). Cotton, sorghum, pearl millet, wheat, groundnut and pulses are the main crops being cultivated in the region. The vegetation of non-agricultural sites consists of short, thorny, thick and whitish-leaved vegetation with scanty trees of *Salvadora persica*.

Sample collection

The soil samples were collected from the non-agricultural fallow region of “L J Dumana Farm, Viramgam, Gujarat” and brought to the laboratory in polythene bags. The samples were from the north, south, east, west and central locations of the field. There was not much natural vegetation and the fields were planted recently with different plant species. The soil was sampled from an upper 5-20 cm depth from the surface of the fallow region of the field. Each soil sample was tagged to differentiate them (Table 1 and Fig. 1). Furthermore, the samples that were collected from the same direction were mixed for better results and named the sample as NM, SM, CM, EM and WM for the north, south, centre, east and west direction, respectively. Samples were stored at 4°C before use for the experiment.

Table 1. Name of the direction, number of samples collected with sample code and region in the field map showing the sampling site

S. No.	Name of the direction	No. of sample collected	Sample code
1	North	5	NN
			NC
			NS
			NE
			NW
			NM
2	Centre	5	CN
			CC
			CS
			CE
			CW
			CM
3	South	5	SN
			SC
			SS
			SE
			SW
			SM
4	East	5	EN
			EC
			ES
			EE
			EW
			EM
5	West	5	WN
			WC
			WS
			WE
			WW
			WM

Isolation of arbuscular mycorrhiza

The wet sieving and decanting procedure (14) was used to isolate and quantify AM spores from 50 g of soil of a separate sample that was taken from "L J Dumana Farm, Viramgam, Gujarat". To break up the clay particles, 500 mL of distilled water were added and the mixture was stirred for 10-15 min while sodium hexametaphosphate was added. To remove spores from organic materials or clay carrier material, a thorough water wash is required. It was left to stand for 2-3 hr. Subsequently, the soil suspension was passed through stacked 400 µm, 250 µm, 100 µm and 25 µm sieves arranged in descending order of size. To ensure that no prospective spores or sporocarps remained in the top sieve, the large particle size on the sieve was transferred to the same beaker in which the soil suspension was formed to repeat the operation. The largest sieve size was at the top and the smallest was at the base. The spores that settled on the surface of the 250 µm, 100 µm and 25 µm mesh sieve were washed and collected in separate beakers. This procedure was repeated 3 to 4 times to collect the maximum spore present in the soil.

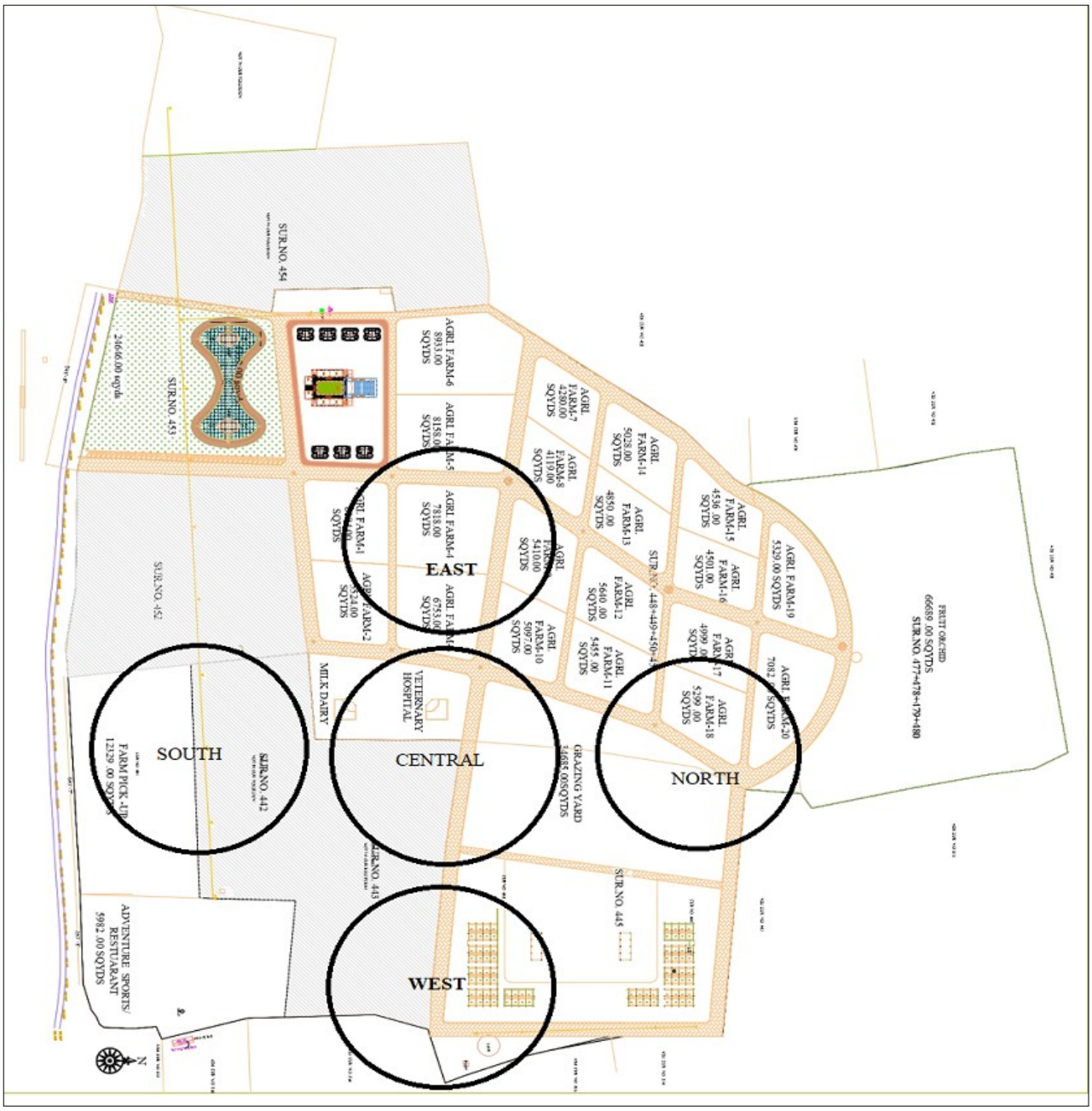


Fig. 1. Field map showing sampling design and sites.

After being sieved, the material was put into centrifuge tubes and whirled in a horizontal rotor for 5 min at 1750 rpm. After carefully decanting the supernatant, the pellet was resuspended in a solution containing 60% sucrose and centrifuged in a horizontal rotor for 5 min at 1750 rpm (15-17). The supernatant (with spores) was poured onto a 25 µm sieve and it was rinsed with water to remove the sugar. The spore with water was collected in a beaker. The water was filtered through the Whatman filter paper. This paper was observed under the microscope and the spores were counted. One end of a matchstick was sharpened and fixed in the needle. The sharpened tip was dipped in lactoglycerol, a single spore was picked up and mounted in lactoglycerol on a clean slide. The spores were observed under the compound microscope at 10 × 40x magnification and a camera phone (android) with autofocus, through-the-lens light meter, minimum × 3 optical zoom and LCD screen was used for photography (18).

Morphological characterization

The manual for the identification of VA mycorrhizal fungus (6), the monograph (19), the INVAM, GINCO, BEG (20-22) websites and the published taxonomic literature were used to characterize the spores for genus and species. Unidentified collections lacked sufficient information to be identified, whereas sp. 1, sp. 2 and so on were collections that had been classified up to the genus level.

Results

Spore count

Table 2 and Fig. 2 show the spore count of mixed samples from different regions (North, Central, South, East and West) of the field. The South region showed the highest spore count among all the regions with 202.00 ± 3.61 spores per 50 g of soil, while the North region had the lowest spore count among all the regions with 80.00±6.56 spores per 50 g of soil.

Table 2. The spore count per 50 g of soil samples and average spore per g of soil at LJ Farm of Dumana, Viramgam, Gujarat

Sample code	Average spore/50 gm soil sample	Average spore/gm soil sample
NM	80.00 ± 6.56	1.60
CM	119.00 ± 6.00	2.38
SM	202.00 ± 3.61	4.04
EM	86.00 ± 6.56	1.72
WM	107.00 ± 9.00	2.14

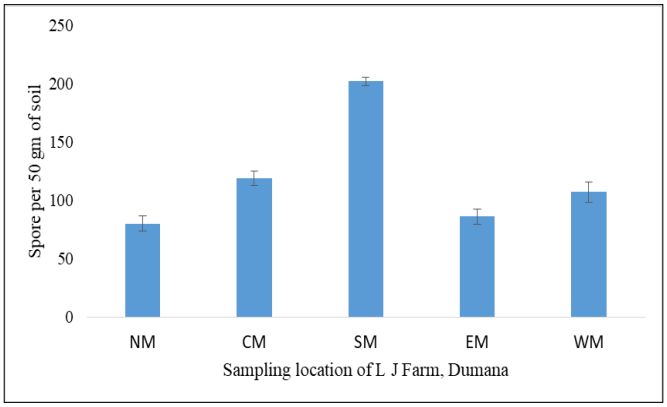


Fig. 2. Bar graph displaying the density of arbuscular mycorrhizal spores in each area of the L J Farm located in Dumana, Viramgam, Gujarat. 'T' represents the ± standard deviation (SD). Sample size of 3 replicates per mixture, each mixture was consisted from 5 sampling sites. NM= north mixed, CM= central mixed, SM= south mixed, EM= east mixed and WM= west mixed.

There was variation in spore counts across different regions, indicating spatial heterogeneity in endomycorrhizal spore distribution in the soil samples. Fig. 3 shows the spore count of samples collected from the north, central, south, east and west regions of the field. There were 5 (NN, NS, NC, NE and NW) sampling sites in the north region. Sample NC had the highest spore count (113 spores per 50 g soil) among all the samples in the north direction, while sample NN had the lowest spore count (42 spores per 50 g soil). The spore count of samples collected from the south region SW had the highest spore count (208 spores per 50 g soil) among all the samples of the south region, while sample SN had the lowest spore count (147 spores per 50 g soil).

In Fig. 3, the spore count of samples collected from the central region CE had the highest spore count (168 spores per 50 g soil) among all the samples of the central region, while sample CW has the lowest spore count (75 spores per 50 g soil). In the spore count of samples collected from the east direction, EC had the highest spore count (160 spores per 50 g soil) among all the samples of the east region, while sample EE had the lowest spore count (74 spores per 50 g soil). The spore count of samples collected from the west region; WW had the highest spore count (153 spores per 50 g soil) among all the samples of the west region, while sample WN had the lowest spore count (75 spores per 50 g soil), respectively.

The sites of higher spore counts were SW (208), CE (168), EC (160), WW (153) and NC (113), whereas the sites of lower spore count in the respective directions were SN (147), CW (75), WN (75), EE (74) and NN (42), respectively.

Spore morphoform & diversity

Different types of spores were isolated from the soil sample collected from L J Dumana farm, Viramgam, Gujarat. All the spores were collected on a 25 µm sieve, so the size of the spores was from

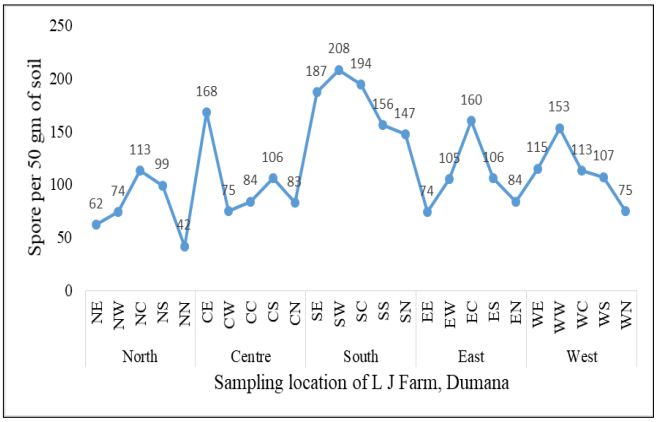


Fig. 3. Graph showing the number of arbuscular mycorrhizal spore count at different sampling site of L J Farm at Dumana, Viramgam, Gujarat. The connecting line is showing the variance in distribution of arbuscular mycorrhizal spore at different location from North to South and East to West.

100 µm to 25 µm. The morphological characteristics, i.e., shape, color, spore surface and presence of hyphal attachment are mentioned in Table 3. From the soil sample collected from LJ Dumana farm, Viramgam, Gujarat, 9 different types of spores were isolated. All these 9 types were observed in composite mixture samples of north (NM), central (CM), south (SM), east (EM) and west (WM) sites. Out of 9 types of spores, two spores were of globular shape, 3 spores were of sub-globular shape and 4 spores were of oval shape. Five spore types had smooth surfaces, whereas 4 spore types had rough surfaces. Four spore types had hyphal attachment, whereas in 5 spore types, no hyphal attachment was observed. A detailed identification key of the isolates is mentioned in Table 3 and Fig. 4.

Spore Morphology

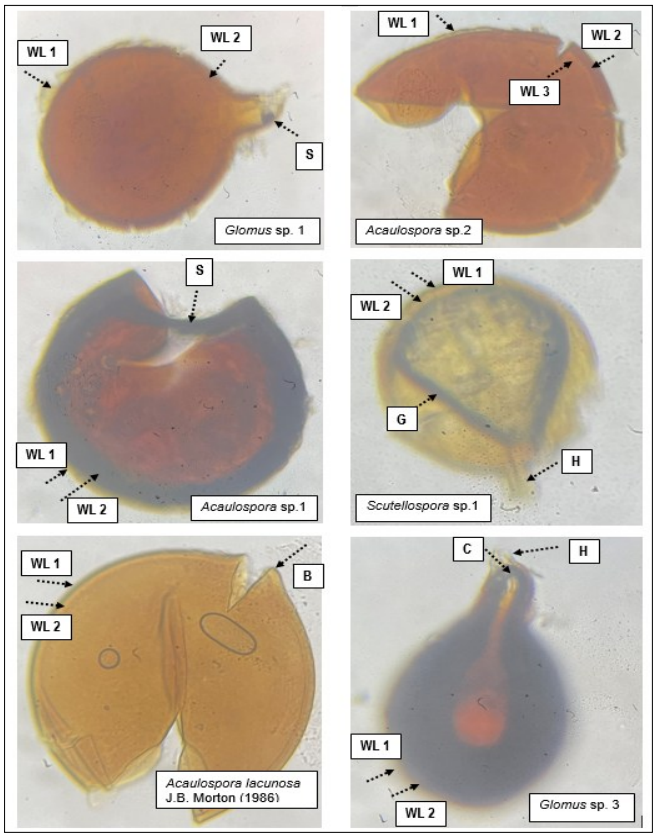
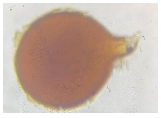
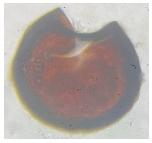
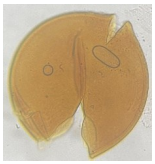
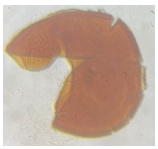
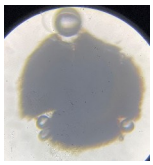
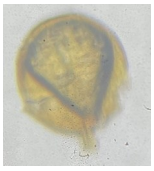
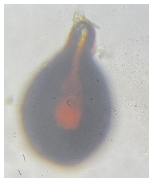
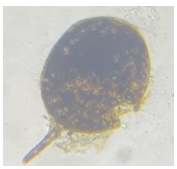
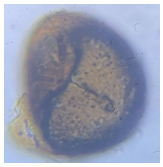


Fig. 4. Wall layers of spores and characteristic structures observed as key for the identification of genera and species of endomycorrhizae. WL 1= wall layer 1, WL 2= wall layer 2, WL 3= wall layer 3, S= septum, G= germination shield, H= hyphae, B= beak like notch and C= septum 'C' shaped giving the appearance of beak like germ tube.

Table 3. Morphological feature of the endomycorrhizal spore, isolated from L J Farm at Dumana, Viramgam, Gujarat

Spore picture	Morphological characters of spores					Genera & species as per key
	Shape	Colour	Spore surface	Hyphal attachment	Wall layer	
	Globular	Brick red	Rough	Present	Wall layer 2, septum straight	*Glomus* sp. 1
	Globular	Reddish brown	Smooth	Absent	Wall layer 2, septum 'C' shaped	*Acaulospora* sp. 1
	Sub-globular	Golden yellow	Smooth	Absent	Wall layer 2–3, beak like notch present	*Acaulospora lacunosa* J.B. Morton (1986)
	Sub-globular	Brick red	Rough	Absent	Wall layer 2–3	*Acaulospora* sp. 2
	Sub-globular	Dark brown	Rough	Absent	Wall layer 2	*Glomus* sp. 2
	Oval	Yellow	Smooth	Present	Wall layer 2, germination shield present	*Scutellospora* sp. 1
	Oval	Reddish brown	Smooth	Present	Wall layer 2–3, septum 'C', giving the appearance of beak like germ tube	*Glomus* sp. 3
	Oval	Yellow brown	Smooth	Present	Wall layer 2 (thin walled), septa straight	*Glomus* sp. 4
	Oval	Yellow brown	Rough	Absent	Wall layer 2, outer wall thin, inner thick	Unidentified

Discussion

The spore count ranges from 42 (minimum) to 208 (maximum) per 50 g of soil. The average count per g of soil varies from 0.84 to 4.16. In the composite mixture of the north (NM), central (CM), south (SM), east (EM) and west (WM), it was 1.60, 2.38, 4.04, 1.72 and 2.14 per g of soil, respectively. Is this density of arbuscular mycorrhiza being good or adequate for sustainable crop production in the semi-arid agro-climatic zone V of Gujarat?. This is a very important question that we would like to address based on the context of our data in comparison with the available literature.

The AMF spore density recorded from 3 sampling sites of the rhizosphere of *Phoenix dactylifera* L in the Kachchh region of Gujarat, which belongs to the same arid agro-climatic region (zone V), was 98, 115 and 251 spores per 100 g of soil, resulting in average spore densities of 0.98, 1.15 and 2.51 spores per g, respectively (23). Additionally, a significant amount of AM spores was found in the rhizosphere soil of grasses from the degraded forest region of Godhra and Baria divisions, located in Gujarat's agro-climatic zone III, approximately 115 km from Vadodara (24). *Heteropogon contortus* rhizosphere soil had 220 spores per 100 g soil, whereas *Themeda triandra* had 165 spores per 100 g and *Chloris barbata* had 150 spores per 100 g, respectively. Rampara soil had 110 spores per 100 g of soil. The average per g spore density in the rhizosphere was 2.2 for *H. contortus,* 1.65 for *T. triandra* and 1.50 for *C. barbata,* whereas Rampara soil was 1.10, respectively.

The distribution of VA mycorrhizal fungi is global and their prevalence is dependent on environmental factors. Even though these fungal plant interactions are extremely important, very little is known about their natural ecology in field soils. Their participation in sustainable agricultural systems necessitates a deeper comprehension (25). The natural occurrence of VA mycorrhizal fungus in soils is influenced by physical, chemical and biological environmental variables as well as soil ecology (26). In the rhizosphere of pigeon pea, chickpea, mung bean, cluster bean and sesbania, the total number of VA mycorrhizal spores in 50 g of soil varied from 10 to 668, 59 to 794, 167 to 235, 98 to 121 and 212 spores, respectively; in contrast, in the rhizosphere of non-legumes, pearl millet, wheat and mustard, the total number of VAM spores in 50 g of soil varied from 65 to 228, 163 to 277 and 130 to 717, respectively (27). The range of spores per 50 g of soil for crops grown in the kharif and rabi seasons was found to be 0-925 and 25-1150, respectively (28). For sorghum, the largest number of AM fungal spores was identified in the rhizosphere soil at 925 spores per 50 g of soil; for cotton, the least number was 25 spores per 50 g of soil; however, for rabi crops, the maximum number was found in the rhizospheric soil of wheat and mustard, with 1150 spores per 50 g of soil.

There is a large variation in the number of endomycorrhizal spores per g of soil. The variation depends on different abiotic, biotic and climatic conditions. The current result shows the number of spores per 50 gm and per gm of soil in a developing plantation field, which was earlier mainly used for the cultivation of local variety of cotton. Since there is no such report on the density and diversity of endomycorrhizae from this region, this primary study will serve as baseline for the comparison of the spore density of future studies of the region/field in different plants and seasons.

Since these fungi cannot be grown on an artificial medium, which is necessary to minimize the effect of abiotic and biotic factors on the morphological traits, taxonomists have difficulty identifying arbuscular mycorrhiza (29, 30). There are no reports of them engaging in sexual activity. Any study on the population structure and diversity of AMF must first clarify the species concept (31). It has also been suggested that since AMF does not have sexual reproduction, applying the species concept may not be practical and that it would be more sensible to base the description of AMF's biodiversity on genetic diversity (32). Over 300 AMF species have been found so far in the world (33–35), whereas 153 species have been reported from India (36). For a group of creatures that is so widely distributed, there may be little taxonomic variety, but there may be significant genetic variability within each species (37). Analysis of the local population's adaptation to varied environments is therefore crucial.

Four species of *Glomus*, 3 species of *Acaulospora*, 1 species of *Scutellospora* and 1 unidentified genus of arbuscular mycorrhiza were characterized from the soil sample of a semi-arid agro-climatic region of L J Farm at Dumana, Viramgam, Gujarat. The genetic structure of AMF, which is multi-genomic and made up of hundreds or thousands of nuclei with varying genetic compositions, is the cause of the functional variety produced by the union of the host plant and AMF. The functional variety of AMF in ecosystems may be enhanced by the genetic variation of nuclei within a single spore, which influences genetic diversity at the population level (38).

Conclusion

The spore density in a developing plantation field that was earlier mainly used for the cultivation of local variety of cotton at Dumana L J Farm Viramgam ranges from 42 (minimum) to 208 (maximum) per 50 g of soil. The average count per g of soil varies from 0.84 to 4.16. In the composite mixture of north (NM), central (CM), south (SM), east (EM) and west (WM), it was 1.60, 2.38, 4.04, 1.72 and 2.14 per g of soil, respectively in agro-climatic zone V of Gujarat. Nine different species of arbuscular mycorrhiza were characterized. *Glomus* and *Acaulospora* were the dominant taxa of the region.

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Authors' Contributions

MIS executed the experimental work and compiled the data, whereas NSS designed the experiment, analyzed the result and prepared the manuscript with discussion. Both authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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