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Research Article
Arbuscular Mycorrhizal Fungi Associated with Rhizosphere of Tomato Grown in Arid and Semi-arid Regions of Indian Desert

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Abstract

Background and Objective: Arbuscular mycorrhizal fungi (AMF) symbiosis is a major survival strategy for plants growing under environmental stresses. Present study aimed at investigating diversity and drivers of AMF in rhizosphere of tomato at arid and semi-arid regions of Indian desert. **Materials and Methods:** Soil and feeder root samples were collected from 24 tomato farms in 6 districts of arid and semi-arid regions of the desert during 2017 cropping season. Spores were analyzed using morphological and molecular (Illumina Miseq sequencing platform) methods. **Results:** About 18 species viz. 8 of *Glomus*, 3 each of *Acaulospora* and *Sclerocystis*, 2 each of *Scutellospora* and *Gigaspora* were isolated. *Glomus mossae, G. intraradices* and *G. fasciculatum* had highest frequency of occurrence (100%) followed by *Gigaspora albida*, *G. margarita* and *A. bireculata* (83% each), while other species ranged between 33-66%. Spore population showed strong positive correlations with root colonization, organic carbon and rainfall, fairly positive correlation with sand, pH, nitrogen and potassium, weak correlations with temperature, silt, clay and electrical conductivity and negatively significant correlation with phosphorous. **Conclusion:** Glomus species were dominant AMF, spore population and root colonization were higher and lower in arid and semi-arid districts respectively, while major drivers of AMF diversity were edapho-climatic factors.

Key words: AMF spores, spore population, glomus species, root colonization, root infection, species diversity, tomatoes

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the arid and semi-arid regions of Indian desert, drought, extreme temperature and progressive salinization of land are among the major abiotic stresses limiting plant growth and productivity[1]. The desert spreads over 1, 96,150 km^2, occupying about 70% of western Rajasthan; lying between 25°45' N latitude and 70°75' E longitude[2]. Today, not only problems of abiotic stresses serious are in these degraded agroecosystems, it seems they are inevitably worsening[3]. Although, plants have a variety of biochemical and physiological mechanisms to cope with adverse environmental conditions, these mechanisms are often not sufficient enough to tolerate elevated and consistent environmental stress situations[4]. Furthermore, a number of conventional strategies such as selection and genetic engineering methods[5], improvement of soil management, irrigation techniques[6] and chemical priming[7] had been introduced to improve stress tolerance in crops in this agriculturally vulnerable ecosystems with little outcomes.

Interestingly, in addition to increasing nutrient and mineral availability and uptake, different mechanisms have been proposed to be involved in tolerance to abiotic and biotic stresses[8] alleviation by AMF plants[6], increased ability for nutrient capturing and cycling and enhancement of plant health through increased protection against pathogen attack. Auge *et al.*[9], further reported that AMF symbiosis is capable of influencing stomatal conductance (g_s) that exerts positive control over water exchange rates and facilitation of water stable aggregates formation[10]. Several findings have also confirmed improved growth performances of AM-colonized salt stressed vegetable crop species such as clover, tomato, sweet basil, cucumber and lettuce[11,12-14]. Thus, a deep understanding of the composition and diversity of AMF in this ecologically stressed ecosystem would serve as a fundamental tool for delving into its functional profile and agricultural importance.

Hitherto, a lot of studies have confirmed the multiple contributions of AMF in the growth and development of most plants under abiotic stresses in wild or agro-ecosystem[15]. However, there is little information on the stress alleviating role of these organisms in the rhizosphere of most vegetable plants such as tomato *(Solanum lycopersicum)* in the arid and semi-arid ecosystem of Indian desert. Tomato is a ubiquitous vegetable, with production and consumption spreading all across the globe, making it one of the best known food ingredients and one of the most beloved vegetables[11]. Because tomato thrives well in warm, sunny conditions with no severe frost, it took well to Indian climate and today, India has become the second largest global producer (next to China) of tomato, producing over 18 million t of tomato annually[16].

Thus, using both morphological and molecular techniques, the present study aimed at investigating the diversity and main drivers of AMF in the rhizosphere of tomato grown in the arid and semi-arid agro-ecological regions of Indian desert. The new knowledge from this study would be a valuable reference for a better understanding of the diversity and unique role of AMF in symbiosis with this important vegetable in the prevailing harsh environmental conditions of Indian desert.

MATERIALS AND METHODS

Study area: Six districts in the arid and semi-arid regions of western Rajasthan were selected for the study during the tomato planting season (May-August, 2017); 4 districts from arid region (Bikaner, Jaisalmer, Barmer and Jodhpur) and two districts from the semi-arid region (Ajmer and Jaipur). From each district, four notable irrigated tomato cultivation fields were chosen for sample collection. All study districts falls within geographical coordinates of 25°-45'N latitude and 70°-75' E longitude. Largest (38,401sq. km) and smallest (22,892 km^2) land areas were found in Jaisalmer and Ajmer districts, respectively. Jaipur district had the highest altitude (487 m) while lowest (229 m) was obtained in Jaisalmer district. Mean monthly temperature was highest in the arid districts (between 45°C in Jodhpur and 49°C in Jaisalmer) while the semi-arid districts recorded 39 and 40°C in Ajmer and Jaipur, respectively. However, average monthly rainfall values in the arid districts were lower (ranging from 209 mm in Jaisalmer to 363 mm in Jodhpur) compared to the semi-arid districts (Ajmer, 557 mm; Jaipur, 601 mm). Important geographical features and climatic variations of the study districts were presented in Table 1 while locations of sample collection were shown in Fig. 1.

Soil sampling and analysis: Sampling was done during the middle of July, 2017 at a depth of 0-30 cm using soil auger after scrapping off the upper layer to remove foreign particles and litters. At least 8 soil and root samples were collected from the rhizosphere of tomato plant at each location and all samples collected from one location were put in sterilized polythene bag, labelled appropriately and mixed to form composite sample for each location. Samples were immediately taken to the laboratory and stored at 4°C for

Fig. 1: Districts showing sample collection sites

1: Kolayat, 2: Nokha, 3: Bikaner, 4: Gersar, 5: Vinjorat, 6: Devikot, 7: Pokaran, 8: Jaisalmer, 9: Bandra, 10: Ranasar, 11: Jalwa, 12: Barmer, 13: Bawari, 14: Mogra, 15: Jodhpur, 16: Bilara, 17: Puskar, 18: Beawar, 19: Ajmer, 20: Kanpura, 21: Shapura, 22: Tala, 23: Chandwaji, 24: Achrol

Source: www.mapsofindia.com

Table 1: Description of study districts

	Districts					
Features	Bikaner	Jaisalmer	Barmer	Jodhpur	Ajmer	Jaipur
Coordinates	28°01N', 73°22'E	26°55N', 70°4'E	25°45'N, 71°25'E	26°17'N, 73°10'E	26°27'N, 74°38'E	26°55'N, 75°46'E
Area (km^2)	30,356	38,401	28,309	22,892	8,481	14,068
Average altitude (m)	237	229	230	268	402	487
Mean rainfall (°C)	325	209	295	363	557	601
Mean temperature (mm)	46	49	47	45	39	40

Table 2: Soil physicochemical characteristics of the study districts

Districts	pH (1:2.5)	EC (dS m^{-1})	OC (%)	AV. P (Mg kg^{-1})	AV. N (Mg kg^{-1})	POT (kg ha^{-1})	SAND (%)	SILT (%)	CLAY (%)
Bikaner	8.50	0.43	0.28	10.7	21.5	324.5	70.2	27.1	2.6
Jaisalmer	8.53	0.55	0.26	8.2	26.7	235.2	70.8	26.4	2.8
Barmer	8.52	0.40	0.25	16.0	16.2	219.2	70.1	27.2	2.7
Jodhpur	8.83	0.60	0.36	19.5	33.0	274.0	67.8	29.3	2.9
Ajmer	8.35	0.67	0.41	33.5	44.0	348.8	56.4	29.1	14.5
Jaipur	8.32	0.63	0.39	30.5	38.7	386.5	57.1	28.2	14.2

EC: Electrical conductivity, OC: Organic carbon, AV.P: Available phosphorous, AV.N: Available nitrogen, POT: Potash

further analysis. Important soil parameters like pH and EC (soil: water ratio), organic carbon content, available nitrogen, available phosphorous, potassium content and soil texture were determined using the standard methods described by AOAC[17]. Values of the analyzed soil parameters were shown in Table 2.

Trap culture: The AMF were amplified using trap cultures due to the characteristic low level of spore densities in desert soils[18]. Five hundred gram dry weight field soil was mixed with autoclaved soil (1:1, v/v) and sown with pre-germinated, healthy and surface sterilized *Triticum aestivum* seeds in pots. All pots were irrigated regularly with sterile water for 45 days in green house at 25±30°C.

Isolation, morphological identification and enumeration of AMF spores: The AMF spores were isolated from rhizospheric soil samples following the Wet-Sieving and Decanting method of Gerdemann and Nicolson[19]. Isolated spores were kept in polyvinyl alcohol, lactic acid mixture in Melzer's reagent and observed under the Olympus CH_2Oi microscope for counting. Counted spores were expressed as number of spores per 100 g of soil. Spore identification was done based on spore size, spore color, wall layers and hyphal attachment using the specifications provided by INVAM[20]. After proper identification, percentage frequency of occurrence was determined using the method of Khade and Rodrique[21].

Assessment of root infection and colonization: Tertiary roots were collected and washed with sterile water to remove all adhering soil debris. The roots were later cut into small pieces of approximately 1 cm length, subjected to differential staining[22] and viewed under dissecting microscope (10 and 40×). Root segments containing vesicles, hartig nets, arbuscules and hyphae of endophytes were considered infected[23]. Root colonization was determined using the grid-line intersect method[24]. The root samples were placed in 7% KOH for 24 h and rinsed in water for clearing. Samples were then acidified in 3.5% HCl for 2 h and stained with 0.05% Trypan blue in 50% glycerol. Root fragments (1 cm) were mounted on slides in glycerol and viewed for intersections under the light microscope. Percentage root colonization was then expressed using the equation described by Philips and Hayman[22] as:

$$\text{Root colonization (\%)} = \frac{\text{No of infected root pieces}}{\text{Total no. of root pieces}} \times 100$$

Molecular analyses: The fast DNA isolation kit (Q-BIOgene; Heidelberg, Germany) was used to extract DNA from 50 g soil sample collected from each location. Extracted DNA were stored at -20°C for PCR reactions. DNA concentration and purity were checked using 1.0% agarose gel. Extracted sequences were amplified using the 18S rRNA gene and primer sets of AMV4.5N Forward 5'-AAGCTCGTAGTTGAATTTCG-3' and AMD R 5'-CCCAACTATCCCTATTAATCAT-3'. The PCR amplification was conducted using extracted method described by Xiao *et al.*[25]. Products of PCR were separated and purified using 1.5% agarose in 0.5×TBE and gel extraction kit (Axygen, Biosciences, NY, USA). The libraries were then sequenced using PE300 sequencing on MiSeq v3 Reagent kit (Illumina) on platform (Illumina, Inc., San Diego, CA, USA). Mothur software application (version 1.33.3) was used to analyze the DNA sequence reads. Aggregation (97%) of decoded information to operational taxonomic units (OTUs) was done using the method described by Edgar[26] and blasting was done using nucleotide collection (nr nt^{-1}) database. The blast hit with highest score was identified as equivalent species.

Data analysis: Operational taxonomic units (OTUs) richness, coverage, Chao's and Shannon's indexes were determined using Mothur software (version 1.33.3) application[27] while indicator species analysis for the locations was determined using the method of Dufrene and Legendre[28]. Analysis of variance (one-way ANOVA) and level of significance ($p<0.05$ and $p<0.01$) were determined using the IBM SPSS Statistics V21×86 model Software Application. Pearson's correlation co-efficient was used to assess the relationship between AMF spore population and various edapho-climatic factors.

RESULTS

Analysis of species diversity, spore population and root colonization: Results of AMF species diversity, spore population and root colonization analysis in the study districts were presented in Table 3. The number of sequence in the soil sample collected from each location ranged from 15238-29147. OTUs coverage in all district locations were up to 99% whilst OTUs number ranged from 45-81 with a genetic distance of 3%. Analysis of Chao's index revealed higher OTUs richness in the arid district (between 70 in Jodhpur and 73 in Jaisalmer) over the semi-arid districts (Ajmer: 58, Jaipur: 57). Similarly, values obtained for AMF diversity using Shannon's index were higher in the arid districts (ranging from 3.13 in Jodhpur to 3.74 in Bikaner) over semi-arid districts (Ajmer: 2.37, Jaipur: 2.74). The AMF spore population values of the studied tomato fields were also higher in the arid district compared to the semi-arid districts and varied between 301

Table 3: Data on sequence analysis, AMF population and root colonization

Districts	Location	No. of sequence	No. of AMF OTUs	Coverage (%)	Chao's index	Shannon's index	AMF population (100 g^{-1})	Root colonization (%)
Bikaner	Kolayat	22,127	66	99	70	3.78	456	48
	Nokha	23,712	79	99	74	3.77	449	49
	Bikaner	22,322	58	99	69	3.67	452	50
	Gersar	22,421	47	99	71	3.74	451	49
Mean					71[a]	3.74[a]	452	49
Jaisalmer	Vinjorat	17,113	51	99	72	3.67	493	55
	Devikot	19,143	49	99	63	3.53	494	53
	Pokaran	16,110	45	99	78	3.88	497	52
	Jaisalmer	15,238	62	99	80	3.55	492	54
Mean					73[a]	3.66[a]	494	53
Barmer	Bandra	21,235	57	99	70	3.71	423	53
	Ranasar	21,561	69	99	68	3.57	420	52
	Jalwa	20,128	76	99	72	3.63	419	53
	Barmer	21,341	63	99	73	3.34	422	48
Mean					71[a]	3.56[a]	421	51
Jodhpur	Bawari	24,176	67	99	68	3.21	383	53
	Mogra	23,124	81	99	72	2.79	381	55
	Jodhpur	23,169	73	99	69	3.45	376	56
Mean	Bilara	24,173	70	99	71	3.08	384	57
					70[a]	3.13[b]	381	55
Ajmer	Pushkar	29,147	69	99	58	2.31	338	64
	Beawar	27,164	57	99	61	2.11	332	66
	Ajmer	28,128	61	99	57	2.53	334	60
	Kanpura	28,263	71	99	56	2.55	330	65
Mean					58[b]	2.37[c]	332	63
Jaipur	Shapura	28,522	62	99	54	2.67	301	61
	Tala	28,115	59	99	57	3.01	299	63
	Chandwaji	27,345	60	99	58	2.98	304	60
	Achrol	28,342	53	99	60	2.87	300	62
Mean					57[b]	2.74[c]	301	61

OTUs defined at cutoff of 3% difference in sequence. Same letter in the same column do not differ significantly at 5% level of probability

(100 g^{-1}) in Jaipur district to 494 (100g^{-1}) in Jaisalmer district. Meanwhile, higher values of percentage root colonization were recorded in the semi-arid districts (Ajmer: 63%, Jaipur: 61%) over the arid districts (between 49% in Bikaner and 55% in Jodhpur).

Assessment of AMF composition and frequency of occurrence: Variation was observed in the AMF composition among the soil samples in all the locations (Table 4). The identified OTUs were affiliated with 5 AMF genera (*Acaulospora*, *Sclerocystis*, *Glomus*, *Scutellospora* and *Gigaspora*) with a total of 18 AMF species. Highest number of species was recorded from *Glomus* (8) which served as indicator species in all the locations. This was followed by *Acaulospora* and *Sclerocystis* with 3 species each, while the genus *Scutellospora* and *Gigaspora* recorded 2 species each. Also, highest percentage frequency of occurrence (100%) was obtained among three species of *Glomus* viz; *G. intraradices*, *G. mossae* and *G. fasciculatum* followed by *Gigaspora* species (*G. albida* and *G. margarita*) and *Acaulospora* species (*A. bireculata*) each with 83% frequency of occurrence. The percentage frequency of occurrence of other AMF species ranged between 33-66%.

Result of correlation between AMF population and edapho-climatic factors: Statistical assessment of the relationship between AMF spore population and edapho-climatic factors of the study areas were presented in Table 5. Results revealed very strong significantly positive correlation between AMF spore population and root colonization (r = 0.913**), annual rainfall (r = 0.812**), organic carbon (r = 0.856**). A good correlation was also observed with available nitrogen (r = 0.719*), available potassium (r = 0.724*), pH (r = 0. 702*) and sand content (r = 706*). The relationships between AMF spore population and EC (r = 0.401), silt (r = 0.231), Clay (r = 0.334) and temperature (r = 0.415) were found to be very weak. However, a significantly negative correlation was observed between AMF spore population and phosphorous content (r = - 0.832**).

Table 4: AMF species distribution, frequency of occurrence (%) and species richness in the study districts

Genus	S/N	Species	Districts						Frequency of occurrence (%)
			Bikaner	Jaisalmer	Barmer	Jodhpur	Ajmer	Jaipur	
Acaulospora	1	*A. rugose*	–	+	–	+	+	–	50
	2	*A. bireculata*	+	+	+	–	+	+	83
	3	*A. strobiculata*	–	–	+	+	+	+	66
Sclerocystis	4	*S. dussii*	–	–	+	+	–	–	50
	5	*S. indica*	+	+	+	+	–	–	66
	6	*S .rubiformis*	+	–	+	+		+	66
Glomus	7	*G. aggregatum*	–	+	+	–	+	–	50
	8	*G. intraradices*	+	+	+	+	+	+	100
	9	*G. deserticola*	+	+	+	+	–	–	66
	10	*G macrosporum*	–	+	+	+	–	–	50
	11	*G. mossae*	+	+	+	+	+	+	100
	12	*G. microsporum*	+	+	–	+	–	+	66
	13	*G. tenerum*	+	+	–	+	–	–	50
	14	*G. fasciculatum*	+	+	+	+	+	+	100
Scutellospora	15	*S. nigra*	+	+	–	–	–	–	33
	16	*S. caulospora*	–	+	+	+	+	–	66
Gigaspora	17	*G. albida*	+	–	+	+	+	+	83
	18	*G. margarita*	+	+	–	+	+	+	83

+: Present, -: Absent

Table 5: Correlation (r) between AMF spore population and various edaphic and climatic parameters

Districts	AMF POP. (100 g)	Root COL. (%)	pH (1:2.5)	EC (dSm)	OC (%)	AV.P (mg kg^{-1})	AV.N (mg kg^{-1})	POT. (kg ha^{-1})	R/FAL (mm)	TEM (°C)	Sand (%)	Silt (%)	Clay (%)
Bikaner	452	49.000	8.500	0.430	0.2800	10.700	21.500	324.500	325.000	46.00	70.200	27.100	2.600
Jaisalmer	494	55.000	8.530	0.550	0.2600	8.200	26.700	235.200	209.000	49.00	70.800	26.400	2.800
Barmer	421	52.000	8.520	0.400	0.2500	16.000	16.200	219.200	295.000	47.00	70.100	27.200	2.700
Jodhpur	381	57.000	8.830	0.600	0.3600	19.500	33.000	274.000	363.000	45.00	67.800	29.300	2.900
Ajmer	332	67.000	8.350	0.670	0.4100	33.500	44.000	348.800	557.000	39.00	56.400	29.100	14.500
Jaipur	301	61.000	8.320	0.630	0.3900	30.500	38.700	386.500	601.000	40.00	57.100	28.200	14.200
Correlation (r)		0.913**	0.702*	0.401	0.856**	- 0.832**	0.719*	0.724*	0.812**	0.415	0.706*	0.231	0.334

*p<0.05, **p<0.01. AMFPOP: Arbuscular mycorrhizal fungi population, ROOTCOL: Root colonization, EC: Electrical conductivity, OC: Organic carbon, AV.P: Available Phosphorous, AV.N: Available nitrogen, POT: Potash, R/FAL: Rainfall, TEM: Temperature

DISCUSSION

The unique ability of plants in the arid zones to release large amount of amino-acids and sugars for AMF growth[29], coupled with the low phosphorous content characteristic of arid soils[2] might have resulted in higher diversity and AMF spore population in arid districts. High-sand content of all soil samples obtained from arid districts (Table 2) might have provided better aeration for soil humus decomposition and hyphae penetration[30] resulting in accelerated fungal propagation and the consequent higher AMF diversity and spore population.

The comparatively higher AMF diversity and spore population recorded in the arid districts (Table 3) might be attributed to the prevalent cultivation practices including intensive irrigation and the use of organic manure. Adequate soil water content had been reported[12] to exert obvious impacts on the distribution of AMF communities by improving the physiological status of local AMF and its ecological niche directly, probably because water is essential for reproductive and metabolic processes. In addition, cultural practice of farmyard manure application by the farmers, might have led to increased level of available nutrients, resulting in elevated number of microbial communities[9].

The maximum percentage root colonization recorded in the semi-arid districts as compared to the arid districts could be due to a combination of factors prevailing in the semi-arid districts viz; higher organic carbon content, optimum levels of nutrients (available N, P and K), favorable temperature and rainfall regimes[6]. In addition, Bhat *et al.*[31] confirmed that there are significant interactions between soil available phosphorous, potassium and AMF root infection and colonization. Soil phosphorous had also been reported[30,32] to stimulate spore germination, hyphal growth and root infection especially under stress conditions.

Conversely, the lower percentage root colonization recorded in the arid districts might be due to effect of

low rainfall and higher temperature. Buenos *et al.*[33] observed that aridity hampered root colonization and reported that in very dry environmental conditions, available water recedes to smaller pores resulting in decreased contact between available spores and water films in the soil. Van der Heidjen *et al.*[34] further reported that lack of soil nutrients inhibited the production and separation of AMF spore for root infection and colonization.

AMF composition and frequency of occurrence: The high species number recorded from the *Glomus* genus (Table 4) in the study area is similar to previously published research[35] that confirmed *Glomus* species to be the most abundant in the AMF assemblage. This could be ascribed to their unique ability to survive in both acidic and alkaline soils, co-adapt with plants to tolerate environmental challenges and produce excellent inoculum under constraint environmental conditions[36].

In addition to its role in carbon allocation, the intermingling and extensive extra-radical mycelium of *Glomus* species also allows for a more efficient exploitation of soil nutrients and water[37], thus benefiting nutrient flow through the soil-fungus-plant system that is particularly relevant in arid ecosystems[33,2]. Querejeta *et al.*[5] also reported that modulation of leaf gas exchange by native, drought-adapted *Glomus* species is critical to the long-term performance of host plants in semi-arid environments. In further work, Querejeta *et al.*[38] hypothesized that enhanced transpiration as well as improved plant water status were key mechanisms involved in plant growth stimulation by native *Glomus* species in the semi-arid soils.

The significantly positive correlation recorded between AMF spore population and organic carbon could be due to the high water and nutrient holding[31,34] and buffering capacities of organic carbon[39-40]. Gerz *et al.*[41] and Bagyaraj and Ashwin[42], observed that AM abundance in the soil could be attributed to the availability or otherwise of its nutrient content which in turn, was credited to the amount of OC, N, P and K among others. Positive correlation observed between AMF spore population with available nitrogen and available potassium in Table 5, agrees with the findings of Timer and Den[29] and Bhat *et al.*[31] but contradicts the results of Khanam *et al.*[43] who reported a negative correlation between soil K and AMF spore population.

CONCLUSION

The present study concludes that (1) Glomus species are the dominant species in the rhizosphere of tomato (*Solanum lycopersicum*) in the studied districts, (2) AMF spore population and root colonization were higher in the arid and semi-arid districts respectively and (3) Edapho-climatic factors have great driving influence on the occurrence and distribution of AMF species in the arid and semi-arid regions of Indian desert.

SIGNIFICANCE STATEMENT

This study provides knowledge on the diversity and drivers of AMF in the ecologically stressed ecosystem of arid and semi-arid regions of Indian desert and reveals promising findings that would provide baseline information for a better understanding of more important roles that AMF plays in enhancing resource allocation and increasing tolerance of tomato plant to the various environmental stresses prevalent in this agro-environment. Findings would also provide researchers with fundamental tools for delving into functional profile of AMF and its symbiotic importance with diverse crops in regions with related edapho-climatic conditions.

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