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Research article

DIVERSITY OF AM FUNGI IN RHIZOSPHERE OF *TRIGONELLA FOENUM-GREACUM* IN WESTERN RAJASTHAN

*Mehboob and Dr. Anil Vyas

Microbial Biotechnology and Biofertilizer Laboratory, Department of Botany, J. N. V. University, Jodhpur, India – 342 003

* Corresponding author: E-mail: mehboob012001@gmail.com

ABSTRACT: A study was conducted to access the diversity of AM Fungi and relationship of *Trigonella foenum-greacum* in Indian Thar Desert. For this purpose, soil samples along with the plant root were collected from different areas of Indian Thar Desert. The survey of AM fungi associated with *Trigonella foenum-greacum* in Indian Thar Desert revealed that eleven AM fungi commonly occur in the rhizosphere viz., *Acaulospora laevis, Acaulospora morrawae, Gigaspora gigangea, Gigaspora margarita, Glomus aggregatum, Glomus constrictum, Glomus deserticola, Glomus fasciculatum, Glomus macrocarpum, Scutellospora calospora* and *Scutellospora nigra*. It was found the all 5 genera of AM Fungi are distributed in these soils. However, relative abundance and qualitative distribution of AM genera was found to vary from place to place.

Key Words: Vasicular Arbuscular Mycorrhiza, *Trigonella foenum-greacum*, Indian Thad Desert.

INTRODUCTION

Trigonella foenum-greacum is extensively grown in tropical and sub-tropical region of India for its vegetable value and seeds, which are medicinally important [1].*Trigonella foenum-graecum* is a good source of dietary protein for consumption by man and animals [2]. Fenugreek is a good soil renovator and widely used as a green manure [3]. Sandy soil with nutrient deficiency, poor water holding capacity and scarcity of water, high temperature with great day-night temperature variations etc. are characteristic features of Indian Thar desert. Due to such harsh climatic condition Indian Thar desert have poor vegetation. AM fungi are obligate symbiotic fungi the hyphae of which develop mycelium, arbuscules and in most fungal genera vesicles in root and is ubiquitous in distribution. These hyphae can explore an area around the root for exceed that available to root hairs. These extramatrical hyphae are more efficient in nutrient uptake than root hairs [4]. Mycorrhizal propagules can survive in the soil as spores which appear to be long-term structures of different ages, states of dormancy and germination periods and they constitute an inoculums source persisting for many years [5]. Arbuscular mycorrhizal Fungi are frequently distributed in different areas of Indian Thar Desert [6]. Manifold beneficial effect of AM fungi includes improved biomass production, nutrient uptake, drought tolerance etc. due to the beneficial effects of AM fungi it is now a day's used frequently in agriculture [7, 8] and forestry. AM fungal colonization aids the host plant in maintaining ionic balance by enhancing and/or selective uptake of nutrients [9]. Studies have shown that arbuscules mycorrhizal associations are beneficial for plants growing in various Indian semi-arid landscapes [10]. The present investigation is based on finding out the relationship between AM fungi and *Trigonella foenum-greacum* and the distribution of AM fungi in mycorrhizosphere of *Trigonella foenum-greacum* in Indian Thar desert.

MATERIAL AND METHODS

Fenugreek plant sample along with rhizosphere soils were collected from six Localities, namely, (1) Bilara (2) Jodhpur (3) Mathania (4) Nagore (5) Osian (6) Tiwari. In each case soil from 10-15 cm depth was dug out.

Trap Cultures

Successive pot cultures (trap cultures) have been shown to be a useful tool in inducing sporulation of AMF from field soils in arid ecosystems to facilitate the detection of AMF species that are present in the rhizosphere and roots but do not sporulate readily in the field at the time of sampling [10,11].

To establish successive pot cultures, 500 g dry wt. field soil was mixed with autoclaved sand (1:1, v/v) and planted with surface-sterilized seeds (by 0.1% w/w mercuric chloride solution for 2 min and then washed with distilled water) of *Cenchrus ciliaris* L. as host.

Root Colonization by AMF

To determine the percent root colonization, root samples collected from different sites were washed in tap water and staining was done by the method of Phillips and Hayman [12] for rapid assay of mycorrhizal association. The root samples were cut into pieces of 1 cm length and placed in 10% KOH solution, which was kept at boiling point for about 10 min (depending upon the hardness of the root sample). The root samples were captured on a fine sieve and rinsed with distilled water until the brown colour disappeared. Post-clearing bleaching was done with alkaline hydrogen peroxide (0.5% NH_4OH and 0.5% H_2O_2 v/v in distilled water). Roots were rinsed with distilled water, treated with 1% HCl and stained with 0.05% w/v trypan blue in lactic acid-glycerol. Assessment of colonization was conducted on each sample by the glass slide method, in which 100 randomly selected root segments of each replication were determined microscopically. A segment was counted as infected when hyphae, vesicles, or arbuscules were observed. The infection percentage was determined by the method given by Giovannetti and Mosse [13].

Spore Extraction

Spores of AMF were extracted from the field and successive pot culture soils by the wet sieving and decanting technique of Gerdemann and Nicolson [14]. Total spore numbers of mycorrhizal fungi in the soil samples were estimated by the method of Gaur and Adholeya [15] and spore densities were expressed as the number of spores per 100 g of soil. The isolated spores were picked up with needle under a dissecting microscope and were mounted in polyvinyl lactoglycerol (PVLG). However, PVLG was mixed with Meltzer's reagent (1: 1, v/v) in case of *Scutellospora* species. All the spores (including broken ones) were examined using Medilux-20 TR compound microscope. Taxonomic identification of spores up to species level was based on spore size, spore colour, wall layers and hyphal attachments using the identification manual of Schenck and Perez [16] and the description provided by the International collection of vesicular and AMF.

Root Staining

Roots staining procedures were adapted from Philips & Hayman [13]. Roots were rinsed to remove adhering soil and debris, and cut into 0.5 to 1 cm long segments, root samples collected were gently washed under tap water, cleared in boiling 10% KOH and stained in lactophenol containing trypan blue.

The percentage of root colonization was calculated by the grid line intersect method [12].

Soil Parameters

Soil samples were analyzed for pH and percentage of soil moisture was calculated on oven dry weight basis. Organic carbon was estimated by the method of Walkley and Black [17] using 1 N potassium dichromate and back titrated with 0.5 N ferrous ammonium sulphate solution.

Site Description

The Indian Thar Desert comprises about 70% part of the Western Rajasthan. Important climatological characteristics of surveyed areas are summarized in Table 1.

RESULT AND DISCUSSION

The Fenugreek plants examined from all the six localities showed well developed VA mycorrhizal association. In all eleven VAM fungi, namely, *Acaulospora laevis, Acaulospora morrawae, Gigaspora gigangea, Gigaspora margarita, Glomus aggregatum, Glomus constrictum, Glomus deserticola, Glomus fasciculatum, Glomus macrocarpum, Scutellospora calospora* and *Scutellospora nigra*. Were collected from different localities from rhizosphere soils of fenugreek.

Physicochemical properties of the soils of each site are presented in Figure 1 & 2. Soil samples collected from rhizosphere soil of *Trigonella foenum-greacum* from various localities showed little variation in different abiotic factors. Soil pH at various localities varied from 6.5 – 8.2, while soil nitrogen was 2.4 – 11.4 mg kg^{-1}. Phosphorus content in soil was almost similar at various localities . Sporulation of different arbuscular mycorrhizal fungi varied from one species to the other. The AM Spore population varied from 310 - 450 spore per 100g soil at various localities. Maximum spore population in *Trigonella foenum-greacum* was observed in case of *Glomus fasciculatum* (55spores/100g soil) followed by *Scutellospora nigra* (45spores/ 100g soil), while *Acaulospora morrawae* showed minimum sporulation (16 spores/100g soil).

Table 1: Site characteristics of surveyed Areas in Rajasthan.

District	Latitude (N)	Longitude (E)	Rain Fall[a] (mm)	Mean Max. Temp (°C)[b]	Mean Min. Temp (°C)[b]
Jodhpur	26°41' 1.8031''	72°53' 55.7048''	389.1	45.1	6.51
Tiwari	26°33' 12.4589''	72°53' 2.5422''	405.5	43.2	5.25
Osian	26°43' 33.6''	72°54' 36''	426.5	45.5	4.23
Bilara	26°10' 44.94''	73°42' 23.2812''	410.3	43.1	5.25
Nagaur	27°11' 55.0644'	73°44' 4.9848'	431.6	42.9	1.44
Mathania	26°31' 47.0604''	72°58' 27.5772''	442.5	44.3	4.25

Table 2. Genera and species of the Glomeromycota found in Different site of western Rajasthan.

Genus	AMF species	Bilara	Jodhpur	Mathania	Nagore	Osia	Tiwari
Acaulospora	*Acaulospora leavis* Gerdmann & Trappe	-	✓	✓	✓	✓	✓
	Acaulospora morrawae Spain & Schenck	✓	-	-	-	✓	✓
Gigaspora	*Gigaspora gigantean* Nicol & Gerd	✓	✓	✓	-	✓	-
	Gigaspora margarita Becker & Hall	-	✓	✓	✓	✓	✓
Glomus	*Glomus aggregatum* Schenck & Smith	-	-	✓	✓	✓	✓
	Glomus constrictum Trappe	✓	✓	-	✓	✓	✓
	Glomus desertocola Trappe Bloss & Menge	✓	✓	✓	✓	✓	✓
	Glomus fasciculatum Gerdmann & Trappe	✓	✓	✓	✓	✓	✓
	Glomus macrocarpum Tul. & C. Tul	✓	-	✓	-	✓	✓
Scutellospra	*Scutellospora calospora* Walker & sanders	-	-	✓	✓	-	✓
	Scutellospora nigra Walker & senders	-	✓	✓	✓	✓	✓

In total, 11 species of Glomeromycota were found in our inveestigation. The maximum number of species was identified in the Osian and Tiwari sites and minimum number of Species was observed in Bilara. *Glomus deserticola*, *Glomus fasciculatum*, *Gigaspora margarita*, *Scutellospora nigra* , and *Acaulospora leavis* were the most dominant species (Table 2). *Glomus* is to be the most abundant of all AMF genera under arid environment [18], which may be due to its resistance to high soil temperature. The density of viable AMF spores recovered from the rhizosphere soil samples collected from field and successive pot cultures were ranged between 20 and 50 spores 10 g^{-1} soil for studied plants. The spore density is relatively low, which is common for arid and semi-arid lands [19]. These findings agree with that of Panwar and Tarafdar [20], who attributes these differences to the length of the growing season and the type of root systems of trees, which make the rhizosphere more favourable to spore propagation and AMF colonization.
In almost all the sites *Glomus* species pre-dominated the AM population and contribute to 25 to 50 percent of the total *Glomus* and were found to be dominant genus Figure – 3.

Other genera found were *Acaulospora*, *Gigaspora* and *Scutellospora*. It is well reported that genus *Glomus* is the most common AMF genus distributed globally and it is also known to dominate in the tropical areas [21] as well as temperate region [22] of the World. Its dominance under various climatic conditions ranging from tropical [23] to high arctic region [24] has been reported earlier. Wide occurrence of genus *Glomus* in the present study as well as reports of several workers suggested that genus *Glomus* has very wide ecological amplitude that is responsible for its adaptability and survival in different habitats and vegetation composition.

AM spore population and percentage of root colonization by different AM fungi in rhizosphere of *Trigonella foenum-greacum* are presented in Figure – 4. *Glomus fasciculatum* colonized root most efficiently (78%) and produced maximum number of spore in the rhizosphere. Mycorrhizal colonization by different AM species at various localities varied from 52–78 %. It was noticed that the species producing maximum spore was also able to colonize the roots most efficiently however, this was not the trend in other mycorrhizal species i.e. *Acaulospora laevis* colonized the root at minimum level while minimum spore population was found in case of *Glomus macrocarpum* hence, spore population could not be corrected with percentage of root colonization [25]. The reason behind this seems to be potentiality of a particular species to colonize the host root rather then the quantity of inoculums. The non-mycorrhizal plants were devoid of mycorrhizal spores in the rhizosphere soil hence there was no root colonization in these plants. The present study reveals the existence of definite AM fungal association in *Trigonella foenum-greacum*. A positive correlation was observed between mycorrhizal spore population and percentage of root colonization in the rhizosphere of *Trigonella foenum-greacum* at various localities. It gives the view that the AM fungi by sporulationg at greater rate also colonizes the host plants most effectively.

Soil pH is the major edaphic factor, which effect the establishment and efficiency of mycorrhizal fungi in natural vegetation. The concentration of soil moisture and organic carbon could not be correlated with AM spore population and root colonization of the *Trigonella foenum-greacum* in this region. The reason for such observation could be very low soil moisture level.

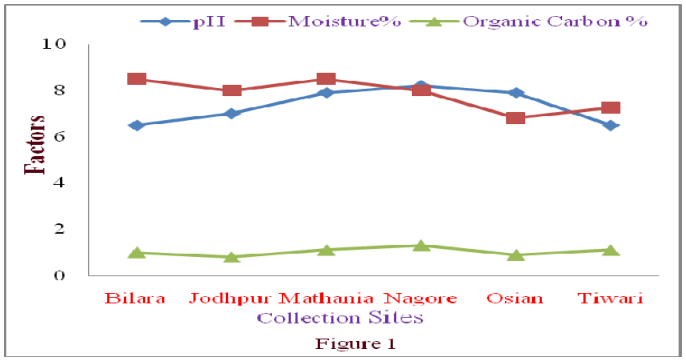

Figure 1: Physicochemical properties of the soils of each site.

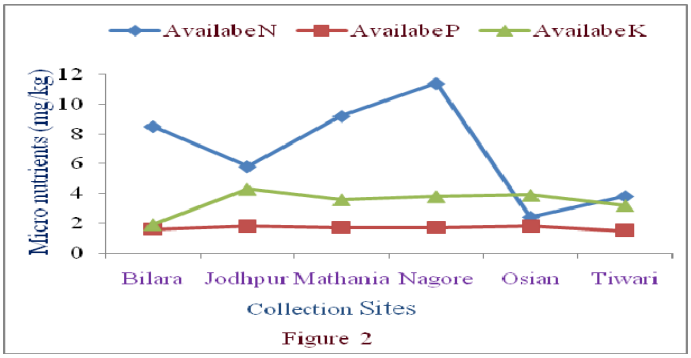

Figure 2 : Physicochemical properties of the soils of each site.

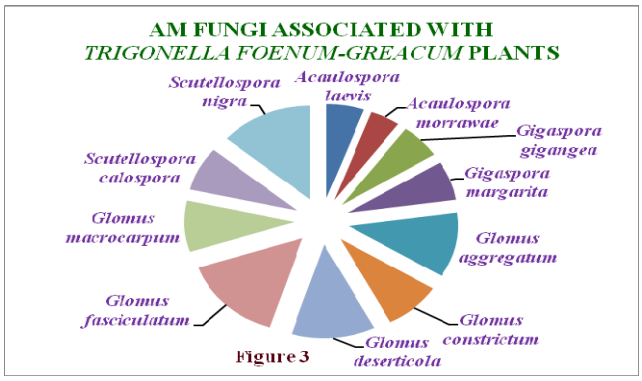

Figure 3: AM Fungal Associated with *Trigonella Foenum Greacum* in Rajasthan.

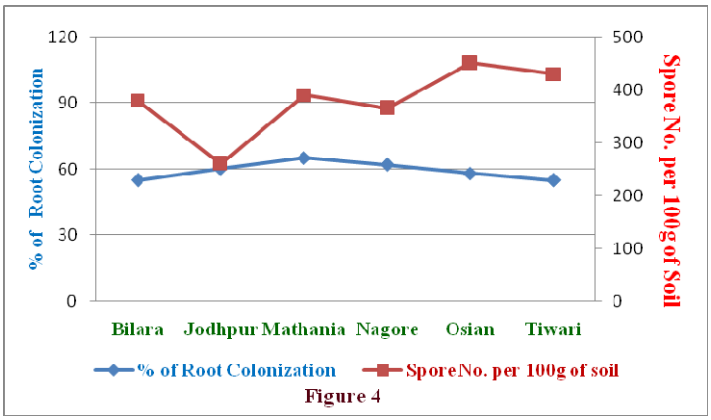

Figure 4: AM spore population and percentage of root colonization.

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