

Diversity of AM Fungi in Rhizosphere of *Calotropis gigantea* in Indian Thar Desert

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Abstract

A study was conducted to access the diversity of AM Fungi and relationship of *Calotropis gigantea* 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 *Calotropis gigantea* in Indian Thar Desert revealed that eleven AM fungi commonly occur in the rhizosphere viz., *Acaulospora laevis*, *Acaulospora morrawae*, *Gigaspora gigantea*, *Gigaspora margarita*, *Glomus aggregatum*, *Glomus constrictum*, *Glomus deserticola*, *Glomus fasciculatum*, *Glomus macrocarpum*, *Scutellospora calospora* and *Scutellospora nigra*. It was found the 4 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.

Keywords: Vascular Arbuscular Mycorrhiza; *Calotropis gigantea*; Indian Thad Desert

1. Introduction

Mycorrhizae occur in a broad range of habitats and ecosystems, are geographically widespread. Arbuscular mycorrhizal fungi are regular component of rhizosphere microflora in natural ecosystem and are necessary for sustainable plant soil systems by establishing symbiotic associations with most land plants and form mycorrhizae. AM fungi inhabit a variety of ecosystems including agriculture lands, forest, grasslands and many stressed environments. The role of mycorrhizae in natural plant population and multispecies communities' remains poorly understood Mathur, and Vyas, (2016). They can modify the structure and function of plant communities Bala, and Mathur, (2015) and may be useful as indicators of ecosystem change Bala, and Mathur, (2013). Arbuscular mycorrhizal Fungi are frequently distributed in different areas of Indian Thar Desert Mathur, et.al.(2009). . Studies on the distribution and activity of AMF can help elucidate the ecological significance of AMF associations. The population of AMF varies greatly and their distribution is affected by various biotic and abiotic factors . Preliminary studies have indicated that AMF are very common in arid soils and form associations with most of the plants growing in Indian desert Mathur, and Vyas, (2000) reported better establishment of vegetation in arid areas by using AMF as these fungi may/often enhance plant absorption of P and other elements, improve water uptake and its transport to plants and enable the plants to withstand high temperatures. Prior to exploiting the biofertilizers potential of AMF in relation to *Calotropis* species, it is necessary to examine the spatial distribution and colonization of these microbes in soil, since AMF species vary with ecosystems (Mathur ,et.al.2010) and are affected by edaphic factors. An extensive field investigation was carried out to evaluate spatial distribution and colonization of AMF species present in the rhizosphere of *Calotropis gigantea* and to study effects of edaphic factors on AMF populations in the rhizosphere.

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2. Material and methods

2.1. Site Description

The Indian Thar Desert comprises about 70% part of the Western Rajasthan, incorporating various districts processing arid and semi-arid regions. Out of which some areas were taken into consideration like Bikaner, Pali, Jaisalmer, Jodhpur, Balotra, An intensive field survey of these sites was undertaken in order to find out occurrence of *Calotropis gigantea* and AMF associations with them. Important climatological characteristics of districts are surveyed.

2.2. Soil Sampling

Rhizosphere soil samples (soil adhering to the roots) were collected at 30-90 cm depths along with root samples in five replicates from *Calotropis gigantea* plants. Before sampling, the soils from the upper layer were scrapped off to remove foreign particles and litter. The collected soil and root samples were placed in an insulated carrier for transport and immediately refrigerated at 4°C upon arrival. The roots were processed immediately. All the soil samples collected from the

rhizosphere of a particular plant species of a district were homogenized replication wise before processing by sieving (< 2 mm mesh size) to remove stones, plant material and coarse roots. Subsample of each soil was air dried and used for estimation of various physico-chemical properties and to establish successive pot cultures (trap cultures).

2.3. 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. 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.

2.4. 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, (1970), 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% NH₄OH and 0.5% H₂O₂ 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 Giovannetti, and Mosse, (1980).

2.5. Spore Extraction

Spores of AMF were extracted from the field and successive pot culture soils by the wet sieving and decanting technique Gerdemann, and Nicolson, (1963). Total spore numbers of mycorrhizal fungi in the soil samples were estimated by method of Mathur, and Vyas, (2000) 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 and the description provided by the International collection of vesicular and AMF.

2.6. Soil Parameters

Soil samples were analysed for pH and electrical conductivity on 1: 2.5, soil: water suspension. Organic carbon was estimated by standard method, using 1 N potassium dichromate and back titrated with 0.5 N ferrous ammonium sulphate solution. Available phosphorus in soil was determined by extraction with 0.5 M sodium bicarbonate for 30 min. Soil texture was estimated gravimetrically by hydrometer method.

3. Results and discussion

An extensive field investigation was carried out to evaluate spatial distribution and colonization of AMF species present in the rhizosphere of *Calotropis gigantea* and to study effects of edaphic factors on AMF populations in the rhizosphere. Table 1. Important climatological characteristics of surveyed districts are summarized in Table 2. Physicochemical properties of the soils of each site are presented in Table 3. Soil texture varies from sandy gravel to clay loam. The soil

had a pH ranged from 6.5 to 8.2, organic carbon between 0.80 and 1.3% and Olsen P level of 38-60 kg/ha. In general, soils are alkaline in reaction, low in organic matter content and available P status.

Table 1 Mycorrhizal spore population and Percentage of Root Colonization in rhizosphere of *Calotropis gigantea* at various Localities

S.No.	Collection site	AM Spore Population (per g soil)	Percentage Root Colonization
1.	Jodhpur	410	78
2.	Pali	380	52
3.	Balotra	430	68
4.	Bikaner	310	54
5.	Jaisalmer	420	73

Table 2 Abiotic Factor of rhizosphere soil of *Calotropis gigantea* at various localities

Sr. No.	Collection site	Soil Moisture %	Soil PH	Soil N k/ha	Soil P k/ha	Organic C%
1.	Jodhpur	8	6.5	20	45	0.8
2.	Pali	8.5	7.9	16	38	1.1
3.	Balotra	6.8	7	23	58	1
4.	Bikaner	7.3	8.2	28	47	0.9
5.	Jaisalmer	8.4	7.5	17	60	1.3

During present investigation AM fungi was found to be scattered over four genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, and *Scutellospora* Table 3. *Glomus* species were most dominant and made up for more than 50% of the total isolates followed by *Acaulospora* (2 species), *Scutellospora* (2 species) and *Gigaspora* (2 species). It is evident that the occurrence of various species of AMF varied considerably with different tree species. In almost all the sites *Glomus* species predominated the AM population and contribute to 25 to 50 percent of the total *Glomus* was found to be dominant genus. Other genera found were *Acaulospora*, *Gigaspora* and *Scutellospora*. It is reported that genus *Glomus* to be the most common AMF genus distributed globally and it is also known to dominate in the tropical areas as well as temperate region of the World. Its dominance under various climatic conditions ranging from tropical to high arctic region 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

This reveals a high specific consortium to each rhizosphere with a high degree of variance in species composition. Hence, a very high AMF diversity index in Thar Desert soils was apparent. *Gigaspora margarita*, *Glomus deserticola*, *Glomus aggregatum*, and *Acaulospora leavis* were the most dominant species Table 3. *Glomus* is to be the most abundant of all AMF genera under arid environment, 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. These findings 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. It is clear from the results that the rhizosphere soils collected from field and successive pot cultures in Jodhpur have higher AMF

spore densities compared to other sites. This may be because of poor soil fertility (in terms of available phosphorus) which results in higher AMF populations. Natural AMF colonization of root samples varied between 38 and 68%. Cleared and stained roots showed the presence of globose to subglobose or ellipsoid bodies (vesicles or spores), dichotomously branched structures (arbuscules) and hyphae in all the sites. Extrametrical hyphae bearing resting spores were also seen associated with the roots of selected plants during study. Considerable variation in percent root colonization and number of different AMF spores associated with plant rhizosphere was observed but no definite correlation could be established between them. However, contradictory results were reported as a significant positive correlation and by, as a negative correlation between percent root colonization and AMF spores. During present investigation observed a significant positive correlation between AMF spore density and soil pH. A positive correlation with organic carbon content in soil coincide with our previous finding. Therefore, may facilitate a more favourable soil moisture condition for the AMF population. When plants have high nutrient availability (especially phosphorus), a negative response and low AMF spore population should be expected. Our results pioneered to identify the status and occurrence of *Calotropis gigantea* and AMF diversity with them, indicating the mycorrhizal dependency of this plant. *Glomus* is considered to be the most common arbuscular mycorrhizal genus in this region. No host plant or geographic location specificity was observed, suggesting the population of AMF species was affected mainly by edaphic factors. Recovery of large AMF diversity with *Calotropis gigantea* reveals the rich wealth of AMF diversity in harsh environmental conditions in Desert. These native AMF isolates with the capacity to survive under stress conditions may be instrumental in the re-establishment of *Calotropis gigantea*. Appropriate strategies can be drawn for the artificial inoculation of one or some of these indigenous AMF, which would make the re-establishment and regeneration attempts ecologically and economically viable in such constrained ecosystems. These approaches will increase our scope to manipulate the symbiosis in conservation scheme.

Table 3 Genera and species of the Glomeromycota found in rhizosphere of at Different sites of *Calotropis gigantea* in western Rajasthan.

Genus	AMF species	Pali	Jodhpur	Balotra	Bikaner	Jaisalmer
<i>Acaulospora</i>	<i>Acaulospora leavis</i> Gerdman & Trappe	-	√	√	√	√
	<i>Acaulospora morrawae</i> Spain & Schenck	√	-	-	-	√
<i>Gigaspora</i>	<i>Gigaspora gigantea</i> Nicol & Gerd	√	√	√	-	√
	<i>Gigaspora margarita</i> Becker & Hall	-	√	√	√	√
<i>Glomus</i>	<i>Glomus aggregatum</i> Schenck & Smith	-	-	√	√	√
	<i>Glomus constrictum</i> Trappe	√	√	-	√	√
	<i>Glomus deserticola</i> Trappe Bloss & Menge	√	√	√	√	√
	<i>Glomus fasciculatum</i> Gerdman & Trappe	√	√	√	√	√
	<i>Glomus macrocarpum</i> Tul. & C. Tul	√	-	√	-	√
<i>Scutellospora</i>	<i>Scutellospora calospora</i> Walker & Sanders	-	-	√	√	-
	<i>Scutellospora nigra</i> Walker & Sanders	-	√	√	√	√

4. Conclusion

The present study reveals association of AM fungi with *Calotropis gigantea* at various localities of western Rajasthan. Four genera of AM fungi showing eleven species were found distributed at various localities in rhizosphere of *Calotropis gigantea* at various localities. Percentage root colonization by AM fungi in roots of the plant varied from 52 to 78 per cent. Edaphic factors were found to affect spore population and root colonization by AM fungi.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by the author.

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