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Arbuscular Mycorrhizal Status of Medicinal Halophytes in Saline Areas of Indian Thar Desert

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Abstract: A field study of 8 saline districts processing salt lakes and salt basins in arid zones of Rajasthan was undertaken to evaluate the occurrence of three selected halophytic plant species (*Suaeda fruticosa* (Linn.) Forsk, *Salsola baryosma* (Roem. et Schult) Dandy, *Haloxylon recurvum* (Moq.) Bunge ex Boiss) and Arbuscular Mycorrhizal Fungal (AMF) associations with them. Five genera were identified in the rhizosphere of these selected plant species. A high diversity of AMF was observed which varied between different host plant species. Among the five genera, *Glomus* occurred most frequently, with eight species, *Acaulospora*, *Gigaspora* and *Scutellospora* were found with three species, respectively, while *Sclerocystis* was detected with two species each. *Gigaspora margarita*, *Gigaspora rosea*, *Glomus deserticola*, *Glomus ambisporum*, *Glomus fasciculatum*, *Glomus mosseae* and *Scutellospora calospora* were the most dominant species. The AMF spore density was not clearly affected by the host plant suggesting that biotic factors may be relatively less important than abiotic/edaphic factors for establishing population pattern. The spore density of AMF had a strong positive correlation with soil pH and organic carbon content and a negative correlation with Olsen's P content of the soil. The association with AMF of these plant species native to the harsh environmental conditions of the Indian Thar Desert may play a significant role in the re-establishment and conservation of these multipurpose halophytic plants.

Key words: Saline districts, halophytes, arbuscular mycorrhizal fungi, abiotic factors, harsh environmental conditions

INTRODUCTION

Suaeda fruticosa (Linn.) Forsk, *Salsola baryosma* (Roem. et Schult) Dandy, *Haloxylon recurvum* (Moq.) Bunge ex Boiss are important halophytic plant species (Chenopodiaceae, shrub) of the saline areas of Indian Thar Desert (Table 1). Huge demand and multipurpose uses of these plants in pharmaceutical industries, population bloom, urbanization, over exploitation and recurring drought and famine in this region make these plant species valuable (Bhandari, 1990; Shetty and Singh, 1991). All these plant species are found in arid regions of Thar desert, which are characterized by poor sandy soils, low organic matter, uncertain and erratic rainfall, high wind velocity and generally experience water deficit during the growth period.

Salinity affects the establishment, growth and development of plants in billions of hectares of earth lands. These alter physio-chemical and metabolic activities of host plant and it is a world wide problem (Giri and Chamola, 2000). In arid and semi-arid areas concentration of salts and availability of water is enough to damage the growth of plant. Major areas of Indian Thar Desert are represented by saline belts of Luni, Pachpadra Pokaran, Sambhar, Baap, Phalodi etc. Large area of this land are

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Table 1: Important uses/medicinal values of the three selected halophytic plants

External morphology of plant	Botanical name	Family	Local name	Uses/medicinal values
A perennial, much branched, erect, glabrous shrub. Branches many, pale, ascending, slender woody, glabrous. Leaves alternate, linear-oblong, or ellipsoid or somewhat obovate, apex obtuse, the floral leaves short, the older leaves deflexed due to development of branches in their axil. Flowers hermaphrodite, solitary or more usually 2-3 in the axil, arranged alternately on long dense spikes.	*Suaeda fruticosa,* (Linn.) Forsk	Chenopodiaceae	Lunaki	Entire dried plant is powdered and taken with honey to cure leucorrhea. Eaten by camels.
A much branched, pale hairy shrub. Stem slender, twiggy. minute alternate, suborbicular, fleshy with membranous margins. Flowers forming dense, cylindrical spikes, solitary in the axil of the leaves.	*Salsola baryosma* (Roem. et Schult) Dandy,	Chenopodiaceae	Lani	Dry powder of leaves is useful to cure Leaves intestinal disorders
A perennial, straggling under shrub, Stem glabrous, pruinose with secretions forming minutely, raised points usually throughout the stem; internodes 1.5-5 cm long; branches divarticate, spreading or recurved. Leaves 3-8 mm long, half terete larger trigouns, obtuse or acute at the apex.	*Haloxylon recurvum* (Moq.) Bunge ex Boiss	Chenopodiaceae	Khar, Lana	Dried powder of leave is given with curd to cure dehydration and loose motions. The ash of the plant is given in water against internal ulcers. It is also used by 'Dhobies' as a substitute for soap.

either barren or are represented sparse vegetation. Arbuscular mycorrhizal fungi (AMF) are a major component of rhizosphere microflora in natural ecosystems, and play significant role in the re-establishment of nutrient cycling in native ecosystems (Peterson *et al.*, 1985). AMF associations are ubiquitous and play an important role in ecosystem diversity. They can modify the structure and function of plant communities (Giovannetti and Gianinazzi-Pearson, 1994; Douds and Millner, 1999) and may be useful as indicators of ecosystem change (McGonigle and Miller, 1996). Studies on the distribution and activity of AMF can help elucidate the ecological significance of AMF associations (Sanders, 1990). The population of AMF varies greatly and their distribution is affected by various biotic and abiotic factors (Mohammad *et al.*, 2003). Preliminary studies have indicated that AMF are very common in arid soils and form associations with most of the plants growing in Indian desert (Kiran Bala *et al.*, 1989; Pande *et al.*, 1999). Hayman (1983) 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. Further, Panwar and Vyas (2002) indicated the significance of AMF in re-establishment and conservation of endangered plants in arid areas. Turnau and Haselwandter (2002) also considered AMF as a tool for re-establishment of endangered plant species. In general, knowledge of the mycorrhization of halophytic plant species is lacking. On the other hand, information of this kind must be considered as a pre-requisite for making plant re-establishment programmes successful (Van der Heijden *et al.*, 1998).

Prior to exploiting the biofertilizers potential of AMF in relation to these halophytic plant species, it is necessary to examine the spatial distribution and colonization of these microbes in soil, since AMF species vary with ecosystems (McGonigle and Miller, 1996) and are affected by edaphic factors (Sanders, 1990).

Keeping these facts in mind, an extensive field investigation was carried out to evaluate spatial distribution and colonization of AMF species present in the rhizosphere of three selected halophytic plants and to study effects of edaphic factors on AMF populations in the rhizosphere.

MATERIALS AND METHODS

Site Description

The Indian Thar Desert comprises about 70% part of the Western Rajasthan, incorporating 8 saline districts processing salt lakes and salt basins viz., Barmer (Pachpadra, Thob and Sanwarla), Bikaner Lunkaransar and Khajuwala), Churu (Tal Chappar), Jaisalmer (Lanela and Pokaran), Jodhpur (Bap and Phalodi), Nagaur (Didwana), Pali (Luni) and Sri Ganganagar (Sakhi). An intensive field survey of these 8 districts was undertaken in order to find out occurrence of selected halophytic plant species i.e., *S. fruticosa, S. barysoma, H. recurvum* and AMF associations with them. Important climatological characteristics of surveyed districts are summarized in Table 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 each plant. 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).

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 (Stutz and Morton, 1996). 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.

Table 2: Important site characteristics of surveyed districts in Western Rajasthan (India)

District	Latitude (N)	Longitude (E)	Total geographical area (sq. km)	Rainfall[a] (mm)	Mean maximum temperature[b] (°C)	Mean minimum temperature[b] (°C)	Relative humidity[b] (%)
Barmer	24°4'-26°32'	70°5'-72°52'	28387	286.7	45.9	3.04	54.7
Bikaner	27°11'-29°03'	71°54'-74°12'	27244	250.9	46.5	2.45	49.6
Churu	27°24'-29°0	'73°44'-75°41'	16830	415.2	47.5	0.20	54.8
Jaisalmer	26°5'-28°0'	69°3'-70°0'	38401	217.4	46.3	2.05	50.2
Jodhpur	26°0'-27°37'	72°55'-73°52'	22850	389.1	46.1	4.51	48.7
Nagaur	26°25'-27°40'	73°1'-75°15'	17718	431.6	42.9	1.44	47.8
Pali	24°45'-26°75'	72°48'-74°20'	12387	427.2	44.8	2.20	55.9
Sri Ganganagar	28°4'-30°6'	72°3'-75°3'	11155	258.1	47.3	1.06	62.2

[a]Average of last 10 years. [b]Average of last 5 years

Root Colonization by AMP

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% 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 Biermann and Lindermann (1981).

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 (1963). Total spore numbers of mycorrhizal fungi in the soil samples were estimated by the method of Gaur and Adholeya (1994) 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 (1990) and the description provided by the International collection of vesicular and AMF (http://invam.caf. wvu.edu).

Soil Parameters

Soil samples were analysed for pH and electrical conductivity on 1: 2.5, soil: water suspension. Organic carbon was estimated by the method of Walkley and Black (1934) 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 (Olsen *et al.*, 1954). Soil texture was estimated gravimetrically by hydrometer method (Jackson, 1967).

Microsoft Excel 2000 was used in the statistical processing of the data (Standard errors of mean. correlation analysis).

RESULTS AND DISCUSSION

During the survey of entire 8 districts (174972 sq. km) it was found that these three halophytic plant species occurred at all the districts. 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 8.10 to 8.42, organic carbon between 0.20 and 0.40% and Olsen P level of 4.2-7.5 mg kg^{-1}. In general, soils are alkaline in reaction, low in organic matter content and available P status.

Nineteen species of AMF were identified in the rhizosphere soils collected from field and successive pot cultures scattered over five genera viz., *Acaulospora, Gigaspora, Glomus, Sclerocystis* and *Scutellospora* (Table 4). *Glomus* species were most dominant and made up for more than 50% of the total isolates followed by *Acaulospora* (3 species), *Scutellospora* (3 species), *Gigaspora* (3 species) and *Sclerocystis* (2 species). It is evident that the occurrence of various species of AMF

Table 3: Physicochemical characteristics of site soils for halophytic plants

District	pH	EC (dSm^{-1})	OC (%)	Olsen P (mg kg^{-1})	Texture
Barmer	8.32±0.01	0.26±0.02	0.45±0.01	5.1±0.03	Sandy gravel
Bikaner	8.34±0.02	0.24±0.01	0.38±0.01	4.9±0.01	Sandy loam
Jaisalmer	8.42±0.03	0.25±0.04	0.38±0.01	4.6±0.01	Sandy
Churu	8.11±0.01	0.22±0.01	0.32±0.01	4.5±0.02	Sandy loam
Jodhpur	8.35±0.02	0.28±0.03	0.38±0.01	4.2±0.04	Loamy sand
Nagaur	8.16±0.01	0.29±0.01	0.20±0.01	5.6±0.01	Loamy sand
Pali	8.18±0.02	0.22±0.02	0.32±0.01	7.7±0.01	Coarse loam
Sri Ganganagar	8.19±0.01	0.18±0.01	0.30±0.01	5.3±0.03	Sandy loam

±Standard error of mean

Table 4: Distribution of AMF species associated with different halophytic plants

	Plant species		
AMF species[a]	SF	SB	HR
Acaulospora laevis Gerdemann and Trappe	++	+++	+
Acaulospora morrawae Spain and Schenck	++	++	-
Acaulospora sporocarpia Berch	-	+	-
Gigaspora margarita Becker and Hall	+++	+++	+++
Gigaspora gigantea (Nicol. and Gerd.) Gerd. and Trappe	-	+	-
Gigaspora rosea Nicol. and Schenck	++	++	+++
Glomus aggregatum Schenck and Smith emend. Koske	+	+	-
Glomus ambisporum Smith and Schenck	+++	+++	+++
Glomus constrictum Trappe	-	+	-
Glomus deserticola Trappe Bloss and Menge	+++	+++	+++
Glomus fasciculatum (Thaxter) Gerd. and Trappe emend. Walker and Koske	+++	+++	+++
Glomus geosporum (Nicol. and Gerd.) Walker	+	++	-
Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe	+++	+++	+++
Glomus sinuosum (Gerd. and Bakshi) Almeida and Schenck	++	+	-
Sclerocystis ceremoides Berk. and Broome	-	+	-
Sclerocystis rubiformis Gerd. and Trappe	++	++	++
Scutellospora calospora (Nicol. and Gerd.) Walker and Sanders	+++	+++	+++
Scutellospora nigra (Red Head) Walker and Sanders	+	+	-
Scutellospora aurigloba (Hall) Walker and Sanders	+	-	-

- = Absent, + = Low (< 20%), ++ = Moderate (20-50%), +++ = High (>50%), SF = *Suaeda fruticosa*, SB = *Salsola baryosma*, H = *Haloxylon recurvum*. [a]Given species were found in at least one site

varied considerably with different plant species. *S. baryosma* colonized with 18 of the total species found associated with selected plants followed by *S. fruticosa* (15) and *H. recurvum* (9). 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*, *Gigaspora rosea*, *Glomus ambisporum*, *Glomus deserticola*, *Glomus fasciculatum*, *Glomus mosseae* and *Scutellospora calospora* were the most dominant species (Table 4). *Glomus* is to be the most abundant of all AMF genera under arid environment (Tarafdar and Kumar, 1996), which may be due to its resistance to high soil temperature (Al-Raddad, 1993).

The density of viable AMF spores recovered from the rhizosphere soil samples collected from field and successive pot cultures were ranged between 84 and 147 spores 100 g^{-1} soil for studied plants (Table 5). However, a considerable variation was found at different study sites (Table 6). The spore density is relatively low, which is common for arid and semi-arid lands (Requena *et al.*, 1996). It is evident from the data that maximum spore densities were recorded for *S. baryosma*, followed by *S. fruticosa* and *H. recurvum*. These findings agree with that of Al-Raddad (1993), 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. It is clear from the results (Table 5) 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 (Norani, 1996).

Table 5: Viable AMF spore population (100 g^{-1} soil) and percent root colonization in rhizosphere or three halophytic plants at different sites in Western Rajasthan (India)

District	AMF spores 100 g^{-1} soil			Percentage root colonization (%)		
	SF	SB	HR	SF	SB	HR
Barmer	85±3.2	116±5.5	84±3.7	56±1.8	42±1.6	39±1.7
Jaisalmer	96±3.6	118±6.4	100±4.3	59±2.4	46±2.2	44±2.2
Jalore	97±4.5	120±6.1	96±3.9	60±2.2	41±1.8	41±2.0
Jodhpur	118±7.1	147±7.3	114±6.0	68±3.1	52±3.0	45±2.9
Nagaur	89±4.2	112±4.6	87±3.9	58±1.9	43±2.6	40±1.7
Pali	90±3.2	92±3.9	94±3.8	61±2.8	46±3.1	41±1.8
Sikar	88±4.4	103±4.5	98±4.1	57±1.8	38±2.4	42±2.3
Sri Ganganagar	91±5.3	99±4.1	88±3.3	61±2.4	40±2.8	43±2.1

±Standard error of mean, SF = *Suaeda fruticosa*, SB = *Salsola baryosma*, H = *Haloxylon recurvum*

Table 6: Spatial distribution of different AMF spores in rhizosphere soils of three halophytic plants

AMF species	Barmer	Jaisalmer	Jalore	Jodhpur	Nagaur	Pali	Sikar
Acaulospora laevis Gerdemann and Trappe	6.8[a]	0.0	4.7	4.4	6.2	4.6	2.6
Acaulospora morrawae Spain and Schenck	0.0	0.0	1.7	0.7	2.7	2.4	2.4
Acaulospora sporocarpia Berch	1.9	1.6	2.1	2.0	1.6	1.9	1.4
Gigaspora margarita Becker and Hall	12.1	11.0	15.0	15.0	11.6	11.9	7.8
Gigaspora gigantea (Nicol. and Gerd.) Gerd. and Trappe	4.0	3.8	0.0	1.4	3.5	3.0	3.5
Gigaspora rosea Nicol. and Schenck	7.6	5.7	3.8	5.3	6.6	5.5	3.3
Glomus aggregatum Schenck and Smith emend. Koske	3.4	3.0	0.0	0.0	3.2	2.2	2.4
Glomus ambisporum Smith and Schenck	6.0	11.0	13.3	12.2	10.7	13.0	11.6
Glomus constrictum Trappe	4.3	3.4	1.7	1.4	4.2	0.0	3.3
Glomus deserticola Trappe Bloss and Menge	11.2	12.8	13.3	16.3	10.7	17.4	10.7
Glomus fasciculatum emend. Walker and Koske	8.7	12.4	10.1	10.2	8.3	7.9	6.5
Glomus geosporum (Nicol. and Gerd.) Walker	4.2	3.5	1.7	0.0	4.5	3.9	1.6
Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe	6.0	11.9	13.3	12.2	5.3	6.5	18.4
Glomus sinuosum (Gerd. and Bakshi) Almeida and Schenck	3.9	2.2	2.3	3.2	3.1	2.8	3.0
Sclerocystis ceremoides Berk. and Broome	4.2	2.5	2.5	0.0	2.7	2.5	2.9
Sclerocystis rubiformis Gerd. and Trappe	2.6	1.9	2.0	1.4	1.7	1.4	1.9
Scutellospora calospora (Nicol. and Gerd.) Walker and Sanders	7.8	10.2	12.5	13.6	9.8	10.9	13.6
Scutellospora nigra (Red Head) Walker and Sanders	5.3	3.1	0.0	0.7	3.6	2.2	3.1
Scutellospora aurigloba (Hall) Walker and Sanders	4.2	3.9	0.0	0.9	3.2	2.8	3.1

[a]Percent values of total spores

Natural AMF colonization of root samples varied between 38 and 68% (Table 5). 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 (Kalita *et al.*, 2002). However, contradictory results were reported by Mutabaruka *et al.* (2002), as a significant positive correlation and by Louis and Lim (1987), as a negative correlation between percent root colonization and AMF spores.

Table 7 reveals correlation analysis between AMF spore population and different edaphoclimatic factors. It is evident from the results that AMF spore populations were affected by soil pH, organic carbon and Olsen P content. A significant positive correlation with pH (r = 0.85, p<0.01) and organic

Table 7: Relationship between AMF spore population and different edaphic factors

Edaphic factors	AMF spore population
pH	0.85**
EC	0.53
OC	0.68*
Olsen P	-0.86**
Temp (max)	0.14
Temp (min)	-0.24
RH	0.08

$*p<0.05; **p<0.01; n = 12$

carbon ($r = 0.68$, $p<0.05$) was recorded during present investigation. In contrast, a strong negative correlation was observed with soil Olsen P content ($r = -0.86$, $p<0.01$). Blaszkowski (1993) while investigating plant communities in Poland, observed a significant positive correlation between AMF spore density and soil pH. A positive correlation with organic carbon content in soil coincide with the findings of Mohammad *et al.* (2003), who reported the same while investigating under semi-arid environment of Jordan. Organic matter content in the soil increases the water-holding capacity of the soil (Brady and Weil, 1996) and, 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 (Dhillon and Zak, 1993).

Our results pioneered to identify the status and occurrence of the selected halophytic plant species in Thar Desert environment as well as AMF diversity with them, indicating the mycorrhizal dependency of these plants. *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 these plants reveals the rich wealth of AMF diversity in harsh environmental conditions in Thar Desert. These native AMF isolates with the capacity to survive under stress conditions may be instrumental in the re-establishment of these halophytic plant species. Thus the present study of mycorrhizal status may well prove crucial for any attempts of re-establishment of such endangered plants. 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 schemes.

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