INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
VOLUME-2, ISSUE-3, JULY-SEPT-2012 ISSN 2231-4490

Coden : IJPAES www.ijpaes.com

Received: 23rd June-2012 *Revised: 26th June-2012* *Accepted: 28th June-2012*

Research article

DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH RHIZOSPHERE OF *CAPSICUM ANNUUM* IN WESTERN RAJASTHAN

Mohnish Vyas* and Anil Vyas

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

Corresponding author- mhnshvs@gmail.com Tel: +919460550302

ABSTRACT: A field survey of various districts of Western Rajasthan was undertaken to evaluate the occurrence of *Capsicum* species and Arbuscular Mycorrhizal Fungal (AMF) associations with them. Four genera were identified in the rhizosphere of *Capsicum* species. A high diversity of AMF was observed which varied between different host *Capsicum* species. Among the genera, *Glomus* occurred most frequently, with species, *Acaulospora*, *Gigaspora* were found with three species, respectively, while *Scutellospora* was detected with two species each. *Gigaspora margarita*, *Gigaspora rosea*, *Glomus deserticola*, *Glomus aggregatum*, and *Acaulospora leavis* were the most dominant species. The AMF spore density was not clearly affected by the host *Capsicum*, 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 *Capsicum* species native to the harsh environmental conditions of the Indian Thar Desert may play a significant role in the re-establishment and conservation *Capsicum* species.
Keywords: Arbuscular Mycorrhizal Fungi, *Capsicum annuum, Glomus, Gigaspora, Acaulospora*, *Scutellospora.*

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 [1]. They can modify the structure and function of plant communities [2, 3] and may be useful as indicators of ecosystem change [4]. Arbuscular mycorrhizal Fungi are frequently distributed in different areas of Indian Thar Desert [5] . 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 [6]. Preliminary studies have indicated that AMF are very common in arid soils and form associations with most of the plants growing in Indian desert [7] 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 *Capsicum* species, it is necessary to examine the spatial distribution and colonization of these microbes in soil, since AMF species vary with ecosystems [8] 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 *Capsicum annuum* and to study effects of edaphic factors on AMF populations in the rhizosphere.

MATERIAL AND METHODS

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, Barmer, Jaisalmer, Jodhpur, Balrawa, Balotra, Chandan,Mathania, Nokha, Pokran, Saina, Samdari.

An intensive field survey of these sites was undertakenin order to find out occurrence of *Capsicum annuum* and AMF associations with them. Important climatological characteristics of districts are surveyed.

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 *Capsicum* 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).

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[9, 10]. 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 [11] 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 [12].

Spore Extraction

Spores of AMF were extracted from the field and successive pot culture soils by the wet sieving and decanting technique [13] Total spore numbers of mycorrhizal fungi in the soil samples were estimated [14] 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 [15] and the description provided by the International collection of vesicular and AMF.

Soil Parameters

Soil samples were analysed for pH and electrical conductivity on 1: 2.5, soil: water suspension. Organic carbon was estimated [16] 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 [17]. Soil texture was estimated gravimetrically by hydrometer method [18].

RESULTS AND DISCUSSION

An extensive field investigation was carried out to evaluate spatial distribution and colonization of AMF species present in the rhizosphere of *Capsicum annuum* 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 8.16 to 8.42, organic carbon between 0.20 and 0.45% and Olsen P level of 4.2-7.7 mg kg^{-1}. In general, soils are alkaline in reaction, low in organic matter content and available P status.

Table 1. Different Attributes of AM Fungal Colonization in the Rhizosphere Soil Collected from the *Capsicum annuum*

Location	No. of vesicles per cm Root Segment	Spore Population per 10 g soil	No. of AM Species	Degree of Colonization (%)
Jodhpur	28	34	10	40
Mathania Jodhpur)	24	27	9	36
Balrawa (Jodhpur)	11.2	27	8	35
Bikaner	18	27	11	37
Nokhra (Bikaner)	15.3	26	9	34
Saina (Bikaner)	12.4	30	8	35
Jaisalmer	12.3	35	9	38
Pokaran Jaisalmer)	20	30	8	26
Chandan Jaisalmer)	22	27	10	31
Barmer	26	32	9	34
Samdari (Barmer)	26	31	8	33
Balotara (Barmer)	20	32	8	32

Table 2: Important site characteristics of surveyed places in Rajasthan (India).

District	Latitude (N)	Longitude (E)	Rain Fall[a] (mm)	Mean Max. Temp (°C)[b]	Mean Min. Temp (°C)[b]	Relative humidity(%)[b]
Jodhpur	26°0'-7°37'	72°55'-73°52'	389.1	46.1	4.51	48.7
Mathania Jodhpur)	26.53631	72.96661	286.7	45.9	3.04	54.7
Balrawa (Jodhpur)	26.33795	73.03055	250.9	43.5	2.45	49.6
Bikaner	27°11'-29°03'	71°54'-74°12'	250.9	46.5	2.45	49.6
Saina (Bikaner)	27.83163	72.4044	217.4	46.3	2.05	50.2
Nokha (Bikaner)	27.50274	73.45574	389.1	46.1	4.51	48.7
Jaisalmer	26°5'-28°0'	69°3'-70°0'	217.4	46.3	2.05	50.2
Pokaran Jaisalmer)	26.86341	71.87959	431.6	48.9	1.44	47.8
Chandan aisalmer)	27.112	71.5525	427.2	46.8	2.20	55.9
Barmer	24°4'-26°32'	70°5'-72°52'	286.7	45.9	3.04	54.7
Samdari (Barmer)	25.80751	72.56146	450.5	47.0	2.50	53.8
Balotara (Barmer)	25.83585	72.2414	240.5	40	3.4	57.4

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

Table 3: Physicochemical characteristics of different district soils of Desert Region

District	pH	EC (dSm-1)	OC (%)	Olsen P (mgkg-1)	Texture
Barmer	8.32	0.26 ± 0.02	0.45 ± 0.01	5.1 ± 0.03	Sandy gravel
Bikaner	8.34	0.24 ± 0.01	0.38 ± 0.01	4.9 ± 0.01	Sandy loam
Jaisalmer	8.42	0.25 ± 0.04	0.38 ± 0.01	4.6 ± 0.01	Sandy
Jodhpur	8.35	0.28 ± 0.03	0.38 ± 0.01	4.2 ± 0.04	Loamy sand

± Standered error of mean

Twelve species of AMF were identified in the rhizosphere soils collected from field and successive pot cultures scattered over four genera viz., *Acaulospora*, *Gigaspora*, *Glomus*, 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* (2 species) and *Gigaspora* (3 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 pre-dominated 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 [19] as well as temperate region [20] of the World. Its dominance under various climatic conditions ranging from tropical [21] to high arctic region [22] 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

Table 4: Distribution of AMF species associated with *Capsicum annuum*

AMF species	***Capsicum annuum***
Acaulospora leavis Gerdmann & Trappe	++
Acaulospora morrawae Spain & Schenck	+
Acaulospora sporocarpia Berch	+
Gigaspora margarita Becker & Hall	++
Gigaspora gigantean Nicol & Gerd	+
Gigaspora rosea Nicol & Schenck	++
Glomus aggregatum Schenck & Smith	+++
Glomus constrictum Trappe	++
Glomus desertocola Trappe Bloss & Menge	+++
Glomus mosseae Gerd. & Trappe	++
Scutellospora calospora Walker & sanders	+
Scutellospora nigra Walker & senders	+

+ = Low (< 20%), ++ = Moderate (20-50%), +++ = High (>50%),

Table 5: Relationship between AMF spore population and different edaphic factors

Edaphic factores	AMF spore population
pH	0.88**
EC	0.53
OC	0.68*
Olsen P	-0.86**
Temp (max)	0.14
Temp (min)	-024
RH	0.08

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

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 deserticola*, *Glomus aggregatum*, and *Acaulospora leavis* were the most dominant species Table 4. *Glomus* is to be the most abundant of all AMF genera under arid environment [23], 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 [24]. These findings agree with that of [25], 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 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 [26].

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 [27] . However, contradictory results were reported by [28], as a significant positive correlation and by [29], as a negative correlation between percent root colonization and AMF spores. Table 5: 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 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 ($r = -0.86$, $p<0.01$). [30,31],while investigating plant communities in that desert, 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 [6],who reported the same while investigating under semi-arid environment of Jordan. Organic matter content in the soil enhances the water-holding capacity of the soil [32] 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.

Our results pioneered to identify the status and occurrence of *Capsicum annuum* 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 *Capsicum annuum* 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 *Capsicum annuum*. 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.

REFERENCES

[1] Sharma, D., R. Kapoor and A.R. Bhaytnagar. 2009. Differential growth response of *Curculigo orchoides* to native AMF communities varying in number and fungal components. European Journal of Soil Biology, 45(4): 328-333.

[2] Finlay, R.D. 2008. Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. J. Exp. Bot. 59 (5): 1115-1126.

[3] Zaller JG , Heigl F , Grabmaier A , Lichtenegger C , Piller K , Allabashi, R., Frank, T. and Drapela, T. 2011. Earthworm-Mycorrhiza Interactions Can Affect the Diversity, Structure and Functioning of Establishing Model Grassland Communities. PLOS ONE 6(12): e29293.

[4] Wu, Y., Jiang, J., Shen, W. and He, X. 2010. Arbuscular mycorrhiza fungi as an ecology indicator for evaluating desert soil conditions. 3(2): 164-170.

[5] Mathur, N. and Vyas, A. 2000. Mycorrhizal dependency of Tamarix aphylla in saline areas of Thar Desert. Naturalia, 25:105-110.

[6] Muhammad, M.J., S.R. Hamad and H.I. Malkawi, 2003. Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. J. Arid. Environ., 53: 409-417.

[7] Mathur, N., Singh, J., Bohra, S., Bohra, A., Solanki, R., Vyas, A. 2009. Synergistic Effect of Inoculation of Plant Growth Promotion Rhizobacteria and Arbuscular Mycorrhizal Fungus on Productivity of Sorghum. J.Mycol Pl Pathol, 39(1): 59-65.

[8] Gai, J. P., Christie, P., Feng, G. and Li, X.L. 2006. Twenty years of research on community composition and species distribution of arbuscular mycorrhizal fungi in China: a review. Mycorrhiza 16: 229–239.

[9] Covacevich, F. and Berbara, R.L.L. 2011. Indigenous arbuscular mycorrhizae in areas with different successional stages at a tropical dry forest biome in Brazil. African Journal of Microbiology Research, 5(18): 2697-2705.

[10] Mathur, N., Singh, J., Bohra, S. and Vyas, A. 2007. Arbuscular Mycorrhizal Status of Medicinal Halophytes in Saline Areas of lndian Thar Desert. International Journal of Soil Science 2 (2): 119-127, 2007

[11] Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.

[12] Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular Arbuscular mycorrhizal infection in roots. New Phytol., 84: 489-500.

[13] Gerdemann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal Endogone species extracted from soils by wet sieving and decanting. Trans. Br. Mycol. Soc., 46: 235-244.

[14] Gaur, A. and A. Adholeya, 1994. Estimation of VAMF spores in soil: A modified method. Mycorrhiza News, 62: 10-11.

[15] Schenck, N.C. and Y. Perez, 1990. Manual for the Identification of VA Mycorrhizal Fungi. 3rd Edn., Synergistic Publications, Gainesville, Florida, USA., pp: 286.

[16] Walkley, A.J. and I.A. Black, 1934. Estimation of soil organic carbon by the chromic acid titration method. Soil Sci., 37: 29-38.

[17] Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean, 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. US Department of Agriculture Circular No. 939. US Government Printing Office, Washington, DC.

[18] Jackson, M.L., 1967. Soil Chemical Analysis. 1st Edn., Prentice Hall of India Pvt. Ltd., New Delhi, India.

[19] Zhao, Z.W., Wang, G.H. and Yang, L. 2003. Biodiversity of arbuscular mycorrhizal fungi in tropical rainforests of Xishuangbanna, southwest China. Fungal Diversity 13: 233-242.

[20] Turk, M.A., Assaf, T.A., Hameed, K.M. and Al-Tawaha, A.M. 2006. Significance of Mycorrhizae. World Journal of Agricultural Sciences 2 (1): 16-20.

[21] Chaurasia, B. 2000. Ecological study of Tropical Forest trees with special reference to Arbuscular Mycorrhizal (VAM) association. Ph.D. Thesis, Dr. H.S.Gour Vishwavidyalaya, Sagar, M.P., India.

[22] Kothamasi, D., Kuhad, R.C. and Babu, C.R. 2001. Arbuscular mycorrhizae in plant survival strategies. Tropical Ecology 42(1): 1-13.

[23] Porras-Alfaro, A., Herrera, H., Natvig, D.O. and Sinsabaugh, R.L. 2007. Effect of Long-Term Nitrogen Fertilization on Mycorrhizal Fungi Associated with a Dominant Grass in a Semiarid Grassland. Plant and Soil, 296(1-2): 65-75.

[24] Barea, J.M., Palenzuela, J., Cornejo, P., Sánchez-Castro, I., Navarro-Fernández, C., Lopéz-García, A., Estrada, B., Azcón, R., Ferrol, N. and Azcón-Aguilar, C. (2011). Ecological and functional roles of mycorrhizas in semi-arid ecosystems of Southeast Spain. Journal of Arid Environments, 75(12): 1292–1301.

[25] Panwar, J. and Tarafdar, J.C. 2006. Arbuscular mycorrhizal fungal dynamics under Mitragyna parvifolia (Roxb.) Korth. in Thar Desert. Applied Soil Ecology, 34: 200-208.

[26] Hamel, C. and Strullu, D.G. 2006. Arbuscular mycorrhizal fungi in field crop production: Potential and new direction. Canadian Journal of Plant Science, 86(4): 941-950.

[27] Kalita, R.K., D.P. Bora and D. Dutta, 2002. Vesicular arbuscular mycorrhizal associations with some native plants. Indian J. For., 25: 143-146.

[28] Mutabaruka, R., C. Mutabaruka and I. Femandez, 2002. Diversity of arbuscular mycorrhizal fungi associated to tree species in semi-arid areas of Machakos, Kenya. Arid Land Res. Manage., 16: 385-390.

[29] Kahiluoto H., Ketoja E., Vestberg M. and Saarela I. 2001. Promotion of AM utilization through reduced P fertilization 2. Field studies Plant and Soil, 231, 65-79.

[30] Panwar, J. and Tarafdar, J.C. 2006. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. Journal of Arid Environments 65, 337-350.

[31] Panwar, J. and A. Vyas, 2002. AM fungi: A biological approach towards conservation of endangered plants in Thar Desert, India. Curr. Sci., 82: 576-578.

[32] Sullivan, P. 2002. Drought Resistant Soil. Fayetteville (AR): Appropriate Technology Transfer for Rural Areas. (www.attra.org/attra.pub/pub/drought.pdf).