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Research article

Diversification of Glomermycota form Arbuscular Mycorrhizal Fungi Associated with Vegetable Crops Cultivated Underneath Natural Ecosystems in Arid Region of Rajasthan, India

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Abstract

An investigation was carried out for twenty-two plant species, cultivated widely as vegetable crops in arid region of Rajasthan, state of India belonged to eight different families to measure their affinity in harbouring symbiotic association with arbuscular mycorrhizal fungi (AM fungi) and nutrient status in rhizospheric soil. Twenty out of the twenty two species were having developed AM fungal colonization in their root tissues with a range of 16.33% to 91.33%. All the soil samples tested were slightly acidic to alkaline and housed AM fungal spore with a density ranges from 80.33-199 spores/ 50g air dried soil. The AM fungal spore density in the soil was not found to have any effect on symbiotic colonization in root tissues of studied vegetable crop plants by Mycorrhizae. The soil chemical analysis was also noticed to have no correlation with both colonization of root tissues by AM fungi and spore densities in the rhizospheric soil. Variations in AM fungal root colonization's and spore densities were found statistically significant. Plant species had a significant role in root tissue colonization by AM fungi.

Keywords: Soil; AM fungi; Vegetables Crop plant; Arid region; Rajasthan

Introduction

Mycorrhizal fungi are important members of the plant microbiome, forming interdependency with the roots of most vascular plants on Earth. Maximum plant species partner with either endomycorrhizal or ectomycorrhizal fungi or these symbioses are thought to represent plant variations to quick and slow soil nutrient cycling rates. Members of the Glomeromycota are responsible for forming mutualistic associations called endomycorrhizae with the roots of more than 90% of the world's vascular plants. These endomycorrhizae are also known as arbuscular mycorrhizal fungi (AM fungi). AM fungi are grouped in a monophyletic group, the phylum Glomermycota. The Glomeromycota were belong to the order Glomerales (Glomales) of the Zygomycota. Glomeromycota form relatively large asexual spores in the soil. Endo and ecto mycorrhizal fungi are reported to beneficial for several host plants in macro and micro nutrients mobilization and uptake, inducing tolerance to drought, soil salinity or alkalinity, and resistance to pathogens [1-5]. Vegetables crops are also reported to have mycorrhizal association [6]. Application of a large amount of inorganic and organic fertilizers to the vegetable field soils also affects the microbial population and their role in nutrient cycling. Soil microbes influence the mutability and uptake of inorganic nutrients by plants. Symbiotic entophytes and mycorrhizae are involved in the uptake of nutrient elements of vital plants. Management of soil microbe is necessary to optimize nitrogen and phosphate nutrition of plants. Before attaining the true management of soil microbes, it is essential to understand better the interactions between plants and microbes in the soil around the roots [7]. There are extensive microbial activities in rhizosphere soil which is colonized by a wide range of microbes having important effects on plant nutrition, overall growth, productivity and soil health. Vesicular arbuscular

mycorrhizal fungi (VAMF) and arbuscular mycorrhizas fungi (AM fungi) are mutualistic symbioses formed between the roots of most plants and fungi [8-12]. Mycorrhizal fungi assist the plants to absorb mineral nutrients from the soil, particularly low available elements such as phosphorus (P), nitrogen (N), potassium (K), iron (Fe), molybdenum (Mo) and cobalt (Co) [13-14]. These fungi are also reported to consistently stimulate plant absorption of zinc and copper and also increase plant resistance to various stresses like water, drought, salt and heavy metal toxicity [13-15]. AM fungi infection enables the most of Cu, Zn and Cd to be retained by roots, allowing less heavy metal translocation to leaves. There is evidence that mycorrhizal plants contain higher concentration of growth hormones than their non-mycorrhizal equivalents [16]. Effective nutrient acquisition by AM fungi is generally attributed to the extensive hyphal growth beyond the nutrient depletion zone surrounding the root [9] and principal avoidance strategies of plants for adaptation to adverse soil conditions is increase in root surface area via mycorrhizae [17].

Mycorrhizae are associated with the roots of most species of angiosperms, gymnosperms, pteridophytes and bryophytes and are important in agriculture, horticulture and forestry. A better understanding of the mycorrhizae of agronomic crops is necessary because of their potential involvement in the sustainable agriculture systems [18]. The abundance and distribution of AM fungi in various plants have been studied in various parts of the world. A number of studies have also been performed on the occurrence and abundance of AM fungi in various plants and the effect of AM fungi on crop plants [19-25]. The present investigation was undertaken to study the AM fungal association in some vegetable crops cultivated in arid zone of Rajasthan state of India on the light of distribution and abundance.

Materials and Methods

Root material and rhizosphere soil sampling

The standard methodology for root and rhizosphere soil sampling was adopted. Soil and root samples of twenty-two vegetable crop plants were collected from various crop fields of different sites of Rajasthan state. Four replications were made for each collection site in case of every sample. Soil samples with roots of respective plant species were collected and placed in plastic bags and tagged for analysis.

Quantification of AM fungal biomass and glomalin stock and pertinent soil quality parameters

Assessment of AM fungal colonization percentage (AMFCP)

Approximately 2.0 g (fresh weight) of fine roots were used for staining and the assessment of AM fungal colonization. Freshly collected roots were thoroughly washed with water several times and cut in to uniform pieces approximately 1cm long. Then the roots were cleared with 10% KOH, acidified with 1 N HCl, and stained in 0.05% Trypan blue [26]. Mycorrhizae infection in roots was expressed as percentage of segments containing fungal structures like mycelia, vesicles and arbuscules.

Assessment of AM fungal spore population (AMFSP)

Air dried soil was taken in four replicates for the determination of spore population. The AM fungal spores were extracted using the modified wet sieving and decanting method of Daniels and Skipper [27] and Gerdemann and Nicolson [28] from rhizosphere soil samples collected from different vegetable species. The contents from all the sieves were taken on filter paper and AM fungal spore density was counted under stereo binocular microscope.

Establishment of monoculture of AM fungal spore

It is a well-known fact that, at a particular time naturally occurring spores may not reflect the actual symbiotic fungal structure and AM fungal species determination often requires more than few healthy spores. For detail understanding of AM fungal composition and their structural adaptations, separate trap cultures were established for identification.

Identification of AM Fungal Species

AM fungal spores were separated under stereo binocular microscope and mounted in PVLG and PVLG Meltzer's reagent. Gentle pressure was applied on coverslip to rupture the spores for details of wall layers. Characterization of individual AM fungal spores was carried out after being subject to morphogenetic and micrometric analysis based on their colour, diameter, shape, wall layers, surface content, hyphal colour, hyphal width, and hyphal attachment with the wall. On this basis, dominant genera of AM fungi were categorized (Table 3). The AM fungi were identified and named made at species level with the help of relevant literature [29-33]. The identity of the AM fungi was further confirmed with the species description of INVAM (http://invam.wvu.edu/).

Physico-chemical analysis of rhizospheric soil samples

Air dried soil samples were used for different physico-chemical analysis. pH was determined in 1.25 (w/v) solutions of samples in water and the same was used for determination of electrical conductivity (EC). Air dried samples was processed (addition of 40% NaoH and distillation) using a Kel Plus Nitrogen estimation system (Class DX, Pelican Equipment's) followed by determination of available nitrogen by titration with 0.02N H_2SO_4 [34]. Available phosphorus was determined by Olsen's method [35] and available potassium was determined in a 1 N ammonium acetate extract using flame photometer [36].

Statistical Analysis

Observations of AM fungal colonization, spore density and physico- chemical properties of vegetable species were analyzed using SPSS (SPSS Inc. version 17.0). Results were subjected to one way analysis of variance and the significant difference was determined according to Duncan's Multiple Range Test at significant level of $P<0.05$.

Results and Findings

Physico-chemical properties of rhizospheric soil samples

pH values of rhizospheric soils were found ranging from slightly acidic to near alkaline (6.50 to 7.53) in a total of 22 angiosperm plant species belonging to eight family, cultivated widely as vegetable crops in arid region of Rajasthan, India (Tables 1,2). Variations in pH, EC, OC and available NKP values among the plant species of all samples was statistically significant at P< 0.05 level (Tables 1,2).

Table 1: Chemical analysis of rhizospheric soils (pH, EC and OC) of different species of vegetables species growing in the arid region of Rajasthan.

Plant Species/Family	Soil pH	EC (dSm-1)	OC (%)
Daucu scarota ssp. Sativa (Apiaceae)	6.50a±0.15	0.123a±0.001	1.62bcd±0.09
Allium cepa L. (Amaryllidiaceae)	6.97ab±0.22	0.123a±0.001	1.41abc±0.10
Allium sativum L.(Amaryllidiaceae)	6.97ab±0.22	0.150abcd±0.021	1.49abcd±0.04
Lablab purpureus (L.) Sweet (Fabaceae)	6.90ab±0.61	0.130ab±0.006	1.62bcd±0.09
Pisum sativum L. (Fabaceae)	6.77ab±0.38	0.131abc±0.003	1.47abcd±0.20
Vigna sinensis Prain (Fabaceae)	7.30ab±0.26	0.164ef±0.035	1.55abcd±0.16
Lycopersicum esculentum L.(Solanaceae)	7.37ab±0.30	0.185abcd±0.30	1.23a±0.12
Solanum melongena Linn.(Solanaceae)	7.6b±0.38	0.136f±0.38	1.33ab±0.06
Solanum tuberosum Linn .(Solanaceae)	7.23ab±0.22	0.198abcd±0.22	1.40abc±0.04
Pomoe abatatas (Solanaceae)	7.23ab±0.18	0.138def±0.18	1.41abc±0.03
Capsicum spp. (annuum)(Solanaceae)	7.23ab±0.18	0.180abcd±0.18	1.44abcd±0.06
Solanumme longina (Solanaceae)	7.07ab±0.17	0.138abcd±0.004	1.45abcd±0.01
Brassica oleracea L. var. Botrytis (Brassicaceae)	7.00ab±0.21	0.134ab±0.006	1.77d±0.06
Brassica oleracea L. var. Capitata (Brassicaceae)	7.00ab±0.15	0.130ab±0.004	1.66bcd±0.06
Raphanus sativus L .(Brassicaceae)	7.17cde±0.28	0.129cdef±0.003	1.70cd±0.14
Abelmoschu sesculentus (Linn) Moench (Malvaceae)	6.83abcd±0.38	0.176abcd±0.020	1.33ab±0.07
Basella alba (Basellaceae)	7.43abcd±0.33	0.156abcd±0.021	1.54abcd±0.17
Cucurbita maxima (Cucurbitaceae)	7.33abcd±0.29	0.134abcd±0.001	1.73cd±0.13
Cucumis sativus(Cucurbitaceae)	7.40abcd±0.25	0.153bcde±0.021	1.63bcd±0.07
Momordic acochinchinensis (Cucurbitaceae)	7.40bcde±0.25	0.171bcde±0.024	1.66bcd±0.06
Momordica charantia L. (Cucurbitaceae)	7.53abcd±0.09	0.138abcd±0.003	1.73cd±0.13
Luffa acutangula L .(Cucurbitaceae)	7.00ab±0.30	0.131abc±0.004	1.73cd±0.13

Values are mean of four replicates. SE-Std error; Values in a column followed by the same letter are not significantly different at P< 0.05 according to DMRT

Table 2: Chemical analysis of rhizospheric soils (Available NPK) of different species of vegetables species growing in the arid region of Rajasthan.

Plant species/Family	Available	Available	Available
	N (%)	P (ppm)	K (ppm)
Daucus carota ssp. Sativa(Apiaceae)	0.0072a±0.00034	8.95a±1.32	212.33a±21.67
Allium cepa L .(Amaryllidiaceae)	0.0067a±0.0001	8.18a±2.17	163.33a±9.39
Allium sativum L. (Amaryllidiaceae)	0.0074a±0.00037	11.07a±0.91	185.00a±26.08
Lablab purpureus (L.) Sweet (Fabaceae)	0.0071a±0.00037	11.07a±0.91	188.00a±23.86
Pisum sativum L. (Fabaceae)	0.0071a±0.00037	9.76a±1.70	186.33a±24.13
Vigna sinensis Prain (Fabaceae)	0.0071a±0.00037	10.02a±0.16	185.00a±22.81
Lycopersicum esculentum L. (Solanaceae)	0.0071a±0.00037	10.62a±1.62	206.00a±26.03
Solanumme longena Linn .(Solanaceae)	0.0067a±0.00031	9.85a±0.29	180.00a±25.01
Solanum tuberosum Linn. (Solanaceae)	0.0074a±0.00037	10.95a±0.86	186.33a±24.13

Pomoea batatas (Solanaceae)	0.0076a±0.00039	10.62a±1.92	185.67a±24.55
Capsicum spp. (annuum) (Solanaceae)	0.0075a±0.00036	11.88a±0.77	212.33a±21.67
Solanumme longina (Solanaceae)	0.0071a±0.00037	10.62a±1.92	185.66a±24.55
Brassica oleracea L. var. Botrytis (Brassicaceae)	0.0073a±0.00036	10.79a±0.93	186.33a±24.13
Brassica oleracea L. var. Capitat a(Brassicaceae)	0.0067a±0.00037	9.51a±1.63	182.00a±25.01
Raphanussativus L.(Brassicaceae)	0.0078a±0.00039	9.85a±1.65	179.34a±25.33
Abelmoschu sesculentus (Linn) Moench	0.0071a±0.00037	9.85a±0.29	186.33a±24.13
(Malvaceae)			
Basella alba (Basellaceae)	0.0074a±0.00033	10.95a±0.86	186.33a±24.13
Cucurbita maxima (Cucurbitaceae)	0.0072a±0.00035	10.70a±0.91	180.00a±25.01
Cucumis sativus (Cucurbitaceae)	0.0067a±0.00037	9.76a±1.70	180.00a±25.01
Momordic acochinchinensis (Cucurbitaceae)	0.0074a±0.00036	10.95a±0.86	186.33a±24.13
Momordica charantia L. (Cucurbitaceae)	0.0071a±0.00037	8.82a±1.86	164.67a±4.33
Luffa acutangula L. (Cucurbitaceae)	0.0072a±0.00039	9.76a±1.70	186.33a±24.13

Values are mean of four replicates. SE-Std error; Values in a column followed by the same letter are not significantly different at P< 0.05 according to DMRT

AM fungal root colonization percentage

Root colonization of AM fungi largely depends on the quantity of AM fungal inoculum potential present within the soil. Though pre-existing hyphae and infected root fragments are shown to successfully colonize the roots of host, germinating spores are considered to be the key players in new host establishment. The distribution and abundance of AM fungal colonization in the root tissues of studied crop plants and AM fungal spore density in the rhizospheric soils of vegetable crops are presented in Table 3. Twenty species of this present investigation out of twenty-two showed their capacity in harbouring mycorrhizal root colonization. The intensity of mycorrhizal fungal colonization in root tissues ranged from 16.33 ± 0.88 to 91.33 ± 2.85 in terms root length examined. The highest root length AM fungal colonization was recorded in Allium sativum L. of Amaryllidiaceae family (91.33a±2.85) and the lowest root colonization in Abelmoschus esculentus (Linn.) Moench of Malvaceae family (16.33a±0.88). The species Brassica oleracea L var. capitata of family Brassicaceae and Basella alba of family Basellaceae appeared to have no mycorrhizal association (Table 3). The AM fungal colonization spectrum of studied families recorded were 41.33%, 85.00-91.33%', 58.67-72.66%, 34.00-56.66%, 0-19.67%, 16.33% and 19.67-77.33% respectively for Apiaceae, Amaryllidaceae, Fabaceae, Solanaceae, Brassicaceae, Malvaceae and Cucurbitaceae. The descending order of eight families in terms of their capacity to harbouring mycorrhizal colonization in average is Amaryllidaceous (81.17%) Cucurbitaceae (61.47%) >Fabaceae (64.55%) >Solanaceae (49.00%) > Apiaceae (41.00%)> Brassicaceae (19.67%) Malvaceae (16.33%)> Basellaceae (0.0%). The AM fungal colonization consisted of fungal hyphae, vesicles, and arbuscules. The percentage of colonization varied among the plant species. A similar study with colonization quantum of 30-68% in case of 21 weed species under 15 families from India was reported by Prasad [21].

Table 3: AM fungi association in roots and spore propagules and species in soil for different vegetable species growing in the arid region of Rajasthan

Plant species	Family	% Infection level	Spores/50g soil	Species of AMF spores
Daucus carota ssp. sativa	Apiaceae	41.33cd±3.67	145.00cd±6.35	Gc, Gi, Gs, Ga
Allium cepa L.	Amaryllidiaceae	85.00j±9.45	168.00e±11.85	Gca, Gmi, Gf, Gi, Gs, Ga
Allium sativum L.	Amaryllidiaceae	91.33a±2.85	190.67f±3.67	Gf ,Gc, Gm, Gs
Lablab purpureus (L.) Sweet	Fabaceae	62.33fg±3.71	166.33e±5.78	Gf, Gm, Gs, Gg
Pisum sativum L.	Fabaceae	72.66jhi±2.85	160.67de±3.28	Gca, Gf, Gc, Gm, Gs, Ga
Vigna sinensis Prain	Fabaceae	58.67fgh±3.71	199.00f±0.58	Ga, Gm, Gs, At
Lycopersicum esculentum L.	Solanaceae	55.33de±5.78	130.67bc±2.40	Gi, Gf, Gs, Gsp, Sc
Solanum melongena Linn.	Solanaceae	34.00bcd±5.77	120.33b±1.45	Ga, Gci, Gm, Gi, Gs, At, Sn

Solanum tuberosum Linn.	Solanaceae	44.33cde±6.06	138.00bc±7.51	Gi, Gmi ,Gf, Gi, Gs
Pomoea batatas	Solanaceae	56.66def±6.67	130.00bc±4.00	Gi, Gs, Gci, Gs
Capsicum spp. (annuum)	Solanaceae	51.66de±6.67	137.00bc±4.04	Ga, Gca, Gs, Sn,
Solanumme longina	Solanaceae	52.00de±3.51	148.00cd±9.07	Ga, Gi, Sn ,Gci, Ga
Brassica oleracea L. var. botrytis	Brassicaceae	0	80.33a±9.60	Gca, Gc, Gf, Gi, Gs
Brassica oleracea L. var. capitata	Brassicaceae	19.66ab±3.84	145.67cd±15.30	Gca Gi, Gc, Gsp
Raphanus sativus L.	Brassicaceae	19.67ab±2.03	133.67bc±6.06	Ga, Gf, Gi, Gca
Abelmoschus esculentus (Linn) Moench	Malvaceae	16.33a±0.88	163.00de±3.51	Gca, Gf, Gi, Gs, Ga
Basella alba	Basellaceae	0	130.33bc±2.73	Ga, Gc, Gs, Gca, Gs
Cucurbita maxima	Cucurbitaceae	74.33ij±6.36	134.33bc±6.06	Gi, Gf, Gs, Gg
Cucumis sativus	Cucurbitaceae	19.67ab±2.03	133.67bc±6.06	Ga,Gc,Gs, Gca
Momordica cochinchinensis	Cucurbitaceae	77.33ij±.78	91.33a±2.85	Gi, Gs, Gci, Al
Momordica charantia L.	Cucurbitaceae	70.33ghi±3.84	126.67bc±3.67	Ga, Gf, Gi,Gmi
Luffa acutangula L.	Cucurbitaceae	65.67gh±0.67	126.67bc±3.67	Gc, Gf, Gs, At

Values are mean of four replicates. SE-Std error; Values in a column followed by the same letter are not significantly different at P< 0.05 according to DMRT

Al- Acaulospora laecunosa; At- Acaulospora tuberculata; Ga-Gigaspora albida; Gc -Glomus constrictum; Gca-Glomus caledonium; Gf -Glomus fasciculatum; Gi -Glomus intraradices; Gm -Glomus mosseae; Gmi - Glomus macrocarpum; Gs - Glomus species; Gci-Gigaspora candida; Gg - Gigaspora gigantea; Gsp - Gigaspora spp.; Sn - Sclerocystis nigra; Sc -Sclerocystis spp.

AM fungal spore population and identification

The Glomeromycota have generally coenocytic mycelia and reproduce asexually through blastic development of the hyphal tip to produce Glomerospores known as AM fungal spores with diameters of 50–500 µm.The collected soil samples for estimation of AM fungal density were of different types and had varying pH values. The mycorrhizal spore population in soil was higher in this pH range, and dominant genera of AM fungi were categorized as Glomus. The concentration of mycorrhizal fungal population in soil ranged from 80.33 ± 9.60 to 199.00 ±0.58. The maximum spore density was recorded in Vigna sinensis of Fabaceae family and the minimum in Brassica oleracea L. Var. botrytis of Brassicaceae family. A variety of spores were recovered from soils and root washings, mainly belonging to the genus Glomus. However, azygospore of Acaulospora and Gigaspora, and sporocarps of Sclerocystis were also recovered, though these were rare.

To facilitate identification, the rhizosphere soil samples of all the vegetable species were processed for isolating different AM fungal propagules. Based on the AM fungal spore characters, the following fungi were identified: two species of Acaulospora, namely, A. Laecunosa and A. tuberculata, six species of Glomus, namely, Glomus constrictum, Glomus caledonium, Glomus fasciculatum, Glomus intraradices, Glomus mosseae, and Glomus macrocarpum; three species of Gigaspora, namely Gigaspora albida, Gigaspora candida and Gigaspora gigantean; one species of Sclerocystis nigra; one unidentified spore each of Gigaspora, Sclerocystis and 17 unidentified spore of Glomus. G. intraradices seems to be the most predominant species, noticed in most of the rhizosphere soil samples of different vegetable species (14 vegetable species) followed by G. fasciculatum (12 vegetable species), G. caledonium (9 vegetable species), G. constrictum (7 vegetable species), Glomus mosseae (5 vegetable species), G. macrocarpum (3 vegetable species) and unidentified Glomus spp. (17 vegetable species). The spore of A. tuberculata was found in three vegetable species namely Vigna sinensis , Solanum melongena and Luffa acutangula while that of A. laecunosa was found only in one vegetable species (Momordica cochinchinensis) in the rhizosphere soil samples. The spore of Gigaspora (G. albida) was noticed in five vegetable species, namely Daucus carota, Allium cepa, Pisum sativum, Solanumme longina and Abelmoschus esculentus followed by G. calaspora was recorded in three vegetable species, namely Pomoea batatas, Solanum melongina and Momordica cochinchinensis while G. gigantea was noticed in two vegetable species of rhizosphere soil samples, namely Lablab purpureus and Cucurbita maxima. The spore of Sclerocystis nigra was recorded only in the rhizosphere soil samples of two vegetable species, namely, Capsicum spp. (annuum) and Solanum melongina and one unidentified species in Lycopersicum esculentum soil sample (Table 3). No definite correlation could be established between AM fungal spore and mycorrhizal root colonization. As multiplication of an endomycorrhiza depends on its association with plant roots, the number of its spores in soils is likely to differ, as shown in the present investigation. The prevalence of AM fungi suggests that the mycorrhizae may be of great importance for plant growth and development in tropical soils, especially in arid and semi-arid zones, which have extremely low soil moisture and are relatively poor in macro and micro nutrients. The activity of mycorrhizae

fungal population, in terms of root colonization and the number of AM fungal spores has been shown to be greatly affected by soil conditions [37-39].

Statistical analyses showed that variations in the amount of mycorrhizal root colonization/infection in various plant species and AM fungal spore density of their rhizospheric soils were significant in most of the cases even at P< 0.005 level. ANOVA showed that plant species played a significant role in the colonization of root tissues by mycorrhizal fungi. Present study is in agreement with the findings of Mosse and Bowen [40], Read et al. [41], Prasad [21], Prasad and Gautam [42] and Pringle et al., [43] Prasad [21] reported a range of 5-370 spores/100g dried soil in India. Comparatively a low spore density like this present study might have been caused by a warmer climate inserting less pressure for sporulation [44] which supports the findings of Nadarajah and Nawawi [45-47] and Prasad [21]. The present study reveals that AM fungi are quite common in most of the vegetable species examined from various places in the arid zones of Rajasthan, India with species of Glomus found to be widely spread among the vegetable of this region. The Glomus species of AM fungi help the various crop plants of the arid region in yield productivity in sustainable manner.

Conflict of Interest Statements

There is no conflict of interest.

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