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SYMBIOSIS OF ARBUSCULAR MYCORRHIZAL FUNGI AND *PENNISETUM GLAUCUM* L. IMPROVES PLANT GROWTH AND GLOMALIN-RELATED SOIL PROTEIN IN BARREN SOIL

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ABSTRACT

The symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and about 90% of all vascular plants provides a direct relation between upper and belowground activities. Arbuscular mycorrhizal fungi (AMF), symbionts with nearly everyone terrestrial plants, produce glomalin-related soil protein (GRSP), which plays a major function in soil structure and quality. Soil aggregation is a mechanism used to engineer the soil environment to maximum water and nutrient use efficiency. This study was conducted to determine the effects of three different native AM fungi i.e. *G. mosseae, G. fasciculatum* and *G. decipiens* either single and in mix inoculation on growth of pearl millet (*Pennisetum glaucum* L.) and glomalin-related soil protein grown in the pot experiment under barren soil conditions. Experimental results showed that AM fungi inoculated plants in barren soil enhance their growth and glomalin related soil protein as compared to non-mycorrhizal treatment. This result reaffirms the prime inevitability of mycorrhiza in semi-arid conditions. Thus the introduction of AM fungi in barren land is a key tool to improve the quality of soil and plant growth.

Keywords: AM (Arbuscular Mycorrizal) fungi, Mycorrhizal symbiosis, Pearl millet (*Pennisetum Glaucum* L.), Plant growth, Glomalin related soil protein (GRSP).

INTRODUCTION

Agricultural land degradation is an increasing worldwide problem that leads to poor soil health. Natural regeneration and revegetation are very slow in a highly disturbed barren soil. Present world demands the production of high quality food a without causing damage of ecosystem [1, 2]. The total land region of Rajasthan is 3,42,239 sq. km out of which 45.25% is characterized as barren land. Rajasthan contributes more than 50% of the cereal is mainly grown on light textured soil having low water holding capacity, poor nitrogen, phosphorus and organic matter content of semi-arid region with limited irrigations [3].

The establishment of plant cover up is the most significant step in re-establishment of barren areas. A lot of researchers have indicated that AM fungi are competent of alleviating the unfavourable effects of drought on plant growth [4, 5].

The symbiotic arbuscular mycorrhizal (AM) fungi have a major impact on the functioning and stability of any ecosystem. AM fungi enhance plant growth directly by providing greater and more efficient access via fungal hyphae for absorption of nutrients, especially P, and delivery of these nutrients to the plants [6]. Arbuscular mycorrhizal (AM) fungi are key organisms of the soil/plant system, influencing soil fertility and plant nutrition, and contributing to soil aggregation and stability by the combined action of extra radical hyphae and of an insoluble, hydrophobic proteinaceous substance named glomalin-related soil protein (GRSP) [7, 8].

Glomalin is defined as organic substance glycoprotein profusely produced by all AMF, measured operationally in soils as glomalin-related soil protein (GRSP) [9]. Glomalin is firmly incorporated into the hyphae and soil in large amounts [10] and is highly positively correlated with soil aggregate stability [11]. Soil aggregates are significant for: (1) maintaining better porosity in soil, which provides airing and water penetration rates good for plant and microbial growth, (2) increasing soil fixity against wind and water erosion, and (3) storing carbon by protecting organic matter from microbial decomposition [12].

However, the relationship between the content of glomalin and other soil properties should be studied. The objective of this study is to identify the relationship of am fungi in plant growth and soil structure improvement glomalin-related soil protein in barren soil.

METHODS AND MATERIAL

Study Sites and Climatic Conditions:

The study area was located in the Jaipur is the capital city of the state of Rajasthan, India is situated in the eastern border of Thar Desert, a semi-arid land (coordinates 26° 55' 19.45'' N and 75° 46' 43.98'' E). The

bio-climate can be described as Mediterranean hot semi-arid type with an average annual rainfall of 650 mm and the elevation above sea level 431 m (1417 ft.). The average of minimum and maximum temperatures ranged between summer 25°C - 45°C and winter 5°C - 22°C respectively during the experimental period [13]. The experiment was set up in the soil collected from barren lands which were subjected to pot trials. The soil samples were collected from both barren sites, i.e. site- I Tonk road and site-II Delhi road located in the Jaipur district (India). The barren soil has loamy sand texture, with the following average initial physicochemical characteristics: pH 8.01; Ec, 0.27 ds/m; organic carbon, 0.10%; nitrogen, 85 kg/ha; phosphorus, 15 kg/ha; potassium, 198 kg/ha; zinc, 0.40 mg kg^{-1}; iron, 3.82 mg kg^{-1}; copper, 0.27 mg kg^{-1} and manganese, 2.64 mg kg^{-1}.

Selection of AM Fungi and Host Plant:

The AM fungi were isolated from the plant roots and their rhizospheric soil of cereals plant cultivated from the field by 'Wet sieving and decanting technique' [14]. Collected VAM fungi spores were identified with the help of identification manual of Scheneck and Perez [15] and Spores of common species of VAM were identified using synoptic keys of the genera and species of Zygomycetous mycorrhizal fungi by Trappe [16]. Three dominant AM fungi were isolated i.e. *Glomus mosseae, Glomus fasciculatum* and *Gigaspora decipiens* for the mass multiplication (starter inoculum). Single and pure culture of every selected dominant AM fungus was raised by "Funnel Technique" of Menge and Timmer [17] using *Sorghum bicolor* as host for two months. Thus mass culture of specified AM fungal species was obtained through pure culturing.

Pearl millet (*Pennisetum glaucum* L.) was chosen as the host plant for this study. In India, bajra (pearl millet) is the essential food crop and use in animal feeds, and more suitable crop in Rajasthan environment [18].

Experimental Setup and Design:

The pot culture experiment was carried out in an "Open air conditions" to know the response of pearl millet (*Pennisetum glaucum* L.) plant with AM fungi (June 2015- September 2015). The pots (25×25 cm) were in use and filled with air-dried sterilized barren soil collected from barren soil of both sites I and II. The pots were filled with 5-10% (w/w) of the inoculums of each AM fungi (alone and combined) as a layer of one- two inches below the soil level and surface sterilized (0.05 % sodium hypochloride) seeds of Pearl millet (*Pennisetum glaucum* L.) were planted [19]. The following treatments were conducted to know the response of VAM on pearl millet the inoculation with alone and combined VAM fungi i.e., T_1- *Glomus mosseae* (single), T_2- *Glomus fasciculatum* (single), T_3- *Gigaspora decipiens* (single), T_4- *Glomus mosseae + Glomus fasciculatum,* T_5- *Glomus mosseae + Gigaspora decipiens* and T_6- *Glomus fasciculatum + Gigaspora decipiens,* T_0- Control (non-inoculated). Three replicates of every treatment were maintained.

Analysis of Various Growth Parameters:

Plants were harvested after 90 days by uprooting them from the each treatment of AM fungi pot trial combination and after harvest the roots, shoots and Ear size and weight were taken separately to determine fresh weight (biomass), and then placed in an oven to dry at 42-45^{0}C to 48 hrs until a constant dry weight was obtained [20]. Mycorrhizal root-colonization percent was studied by 'Rapid clearing and staining method' of Phillips and Hayman [21].

Extraction and Determination of Glomalin related soil protein (GRSP):

Glomalin related soil protein (GRSP) extractions were by the procedure described by Wright and Upadhyaya [22, 23].

One-gram sample of air-dried soil from each treated pots were placed in 8 ml 20 mM citrate, pH 7.0 and were autoclaved (121 ^{0}C) for 30 minutes. Then the samples tubes were followed by centrifugation at 10,000 x g for fifteen minutes to pellet the soil particles. The supernatant (easily-extractable glomalin, EEG) was decanted and stored at 4^{0}C. Next, in the same pellet soil sample was used with 50mM sodium citrate, pH 8.0 and repeated autoclave at 121 ^{0}C for 60 minutes. After autoclave cycle, the sample was centrifuged at 8000 x g for 15 minutes. The supernatant total glomalin (TG) was decanted straw/ red-brown colour [23]. The extraction process was continued until the supernatant were become almost colorless and the supernatant was stored at 4 °C before measuring [24]. The supernatant was determined for glomalin related-soil protein (EEG and TG) by the Bradford assay with bovine serum albumin standard. Based on the total volume of the supernatants collected, TG and EEG concentrations were extrapolated into mg g^{-1} of the soil.

Qualitative Analysis (Techniques for Visualizing Proteins) of Glomalin through SDS PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis):

Because of the nature of this glycoprotein (high stability and hydrophobicity), it is difficult to solubilize and to digest for electrophoretic analysis. In addition, its high lectin binding ability prevents it from being stained with simple staining procedures, such as the use of Coomassie Blue. Qualitative analysis of extracted glomalin related soil protein (GRPS) was through SDS PAGE (12.5%) techniques [22]. Protein bands on the gels were visualized by the silver staining method. Gels were scanned and acquired image were analyzed using Quantity One 2D Bio-Rad software.

Statistical Analysis:

All determinations of plants growth parameters and the measurements of glomalin-related soil protein were conducted using 3 replicates (n=3). The value for each sample was calculated as the mean ± SD

Statistical analyses was carried out using Microsoft Excel 2007.

RESULTS AND DISCUSSION

In this study, inoculation with AM fungi provided an important improvement to yield. The data obtained from pot experiment showed that *G. mosseae* and *G. fasciculatum* inoculation on pearl millet plant was showed significant increase in terms of shoot-root length, fresh and dry weight (biomass), mycorrhizal colonization and glomalin-related soil protein as compared to their non-mycorrhizal controls under barren conditions.

A. Growth attributes

The plant height and biomass (fresh/dry) of pearl millet were significantly influenced by AM fungi inoculation. The different AM fungi treated host plants showed increased level of plant total height and percent of mycorrhizal-root colonization. It was noticed that, in this present study the highest plant height 170.73 cm was noted in *G. mosseae* and *G. fasciculatum* fungi (T_4) site–II treatment followed by 165.36 cm of *G. mosseae* (T_1) treatment plants of Site –I compared to control (T_0).

S. No.	Treatments	Plant Length						Mycorrhizal Colonization %	
		Root Length (cm)		shoot Length (cm)		Ear Length (cm)			
		Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II
1	Control (T_0)	13.06±1.00	12.36±0.55	73.93±1.67	73.33±1.52	12.83±0.28	12.26±0.70	00.0±00	00±00
2	T_1	21.36±1.09	20.36±1.51	126.16±3.40	128.33±3.51	17.73±0.75	16.67±0.92	94.6±3.33	90.4±2.38
3	T_2	20.23±0.68	19.43±0.51	108.23±4.61	121.66±3.54	17.13±1.02	15.50±0.50	88.3±1.95	89.2±2.80
4	T_3	18.86±1.02	17.26±1.10	96.33±3.05	94.66±2.25	15.00±0.50	13.75±0.25	61.5±1.80	53.3±2.41
5	T_4	20.20±0.72	19.36±0.40	134.33±3.78	129.73±2.36	16.20±0.20	15.55±0.25	92.1±2.19	89.5±2.58
6	T_5	18.10±0.36	17.93±0.50	114.83±1.04	113.40±3.00	15.90±0.17	15.35±0.35	84.1±1.15	83.0±2.00
7	T_6	17.20±1.05	17.30±0.43	112.23±0.68	98.56±1.50	15.10±1.01	14.33±0.61	81.0±1.90	76.8±2.36

Data represents an average of 3 replicates indicates ± SD, T- treatment with diff. VAM fungi sps.,

Table 1: Morphological parameters of Site-I and site-II pot trials summer crop (pearl millet) plants

B. Mycorrhizal-Root Colonization (%)

The treatments with AM fungal inoculation showed significant increase in root colonization over control. The root colonization percentage was observed to be highest in T_1 (94 %) followed by T_4 (88%) (Site

I) performed on par with the control table-1.

Sample Site	Parameters / Treatments	Plant Fresh Weight (g)				Plant Dry Weight (g)			
		Leaf	Stem	Root	Ear	Leaf	Stem	Root	Ear
Site I	Control (T_0)	13.13±1.87	30.65±1.54	9.12±0.44	6.83±0.90	3.58±0.06	17.38±0.04	2.89±0.03	2.89±0.07
	T1	18.83±0.95	53.92±1.35	17.40±0.84	14.84±0.76	4.99±0.57	30.60±1.43	5.09±0.77	5.93±0.69
	T2	16.30±0.70	53.18±0.60	13.96±0.49	11.93±0.66	4.75±0.51	32.31±0.99	5.11±0.75	5.28±0.17
	T3	13.81±0.87	43.43±1.59	11.51±0.89	8.49±0.31	3.97±0.33	24.12±1.14	3.89±0.67	3.54±0.23
	T4	18.42±0.31	53.19±1.51	16.06±0.25	13.86±0.29	5.20±0.24	30.10±1.67	4.71±0.49	5.71±0.24
	T5	17.23±0.45	49.63±0.40	14.41±0.84	9.46±0.48	4.46±0.37	29.72±0.14	4.32±0.43	4.29±0.28
	T6	14.97±0.75	47.91±0.97	12.31±0.70	9.03±0.11	4.29±0.47	28.21±0.95	3.61±0.46	4.10±0.13
Site II	Control (T_0)	14.33±0.72	25.94±1.96	7.88±0.68	7.05±0.52	4.14±0.03	14.44±0.64	3.01±0.16	2.84±0.23
	T1	18.32±0.49	53.47±0.49	15.81±0.55	14.59±0.71	5.11±0.14	30.27±0.33	4.77±0.18	6.26±0.75
	T2	15.65±0.77	52.73±0.58	13.47±0.48	11.62±0.45	5.19±0.52	30.31±0.32	4.50±0.39	5.18±0.16
	T3	13.40±0.44	43.74±1.84	10.32±0.69	8.28±0.29	3.62±0.39	24.43±1.16	3.40±0.40	3.55±0.13
	T4	18.03±0.25	52.99±1.11	15.86±0.55	14.37±0.74	5.06±0.11	29.35±1.04	5.05±0.21	5.14±0.53
	T5	17.16±0.40	49.40±1.32	14.31±0.43	9.29±0.39	4.51±0.26	28.56±1.44	4.32±0.22	4.11±0.04
	T6	14.28±0.40	46.34±0.63	11.23±0.50	8.38±0.45	4.14±0.10	26.55±0.42	3.70±0.54	3.88±0.13

Data represents an average of 3 replicates indicates ± SD, T- treatment with diff. VAM fungi sps.,

Table 2: Biomass (fresh/dry weight) of Site-I and site-II pot trials summer crop (pearl millet) plants

C. Glomalin-related Soil Protein

The highest Glomalin related soil protein (Total glomalin 3.01 mg g^{-1} and easily extractable glomalin 1.08 mg g^{-1}) was found in inoculation with *Glomus mosseae* (T_1) respectively (site I) pot trials soil.

Qualitative analysis of purified glomalin through SDSPAGE: The protein bands detected and resolved using electrophoresis technique illustrated the molecular mass of the protein to be approximately 65 kDa (60 - 65

kDa) in the AM fungal treatments T_4 (combined inoculation G.M and G.F) Figure-1. Banding patterns also showed T1, T2, T_3, T_5 and T_6 with marked expression of glomalin protein and this was in conformity with results of a study which showed prominent bands of glomalin at 60 kDa to 65 kDa.

S. No.	Treatments	Glomalin (mg/g)			
		EEG		TG	
		Site I	Site II	Site I	Site II
1	**Control (T_0)**	0.20±0.03	0.18±0.02	0.29±0.01	0.25±0.05
2	**T1**	1.08±0.10	0.98±0.08	3.01±0.03	2.91±0.10
3	**T2**	1.02±0.07	0.96±0.02	2.95±0.04	2.88±0.14
4	**T3**	0.63±0.06	0.62±0.09	2.14±0.17	1.93±0.17
5	**T4**	0.99±0.06	0.96±0.03	2.92±0.11	2.89±0.10
6	**T5**	0.90±0.06	0.85±0.07	2.68±0.19	2.15±0.18
7	**T6**	0.80±0.05	0.78±0.05	2.29±0.17	2.09±0.14

Table 3: Glomalin-related soil protein TG (Total glomalin) and EEG (Easily extractable glomalin) of Site (I) and Site (II) pot trials soil

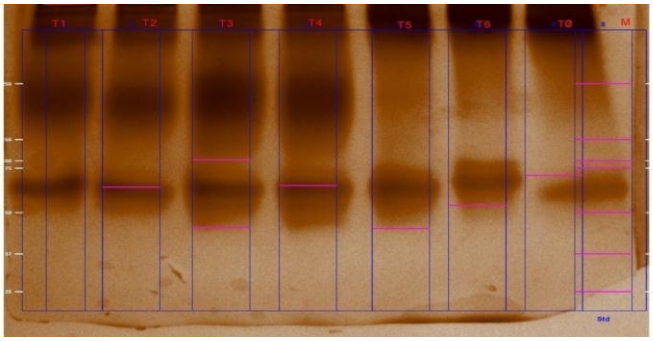

Figure 1: Glomalin protein Banding patterns showed in SDS page with silver stain (MW ~ 60-65 kDa)

The improvement of mycorrhizal plant water relations in turn affected plant growth, such as shoot dry weight and root dry weight. The AM fungal species of *Glomus mosseae* –inoculation was showed higher plant biomass and mycorrhizal development than other two fungal treatments; the result is in agreement with the finding of Jayne and Quigley [25]. This results of the study were consistent with previous reports of Borde *et al.* [26] the reported that growth of mycorrhizal and non-mycorrhizal *Pennisetum glaucum* (L.) crop under salinity stress condition. Therefore, mycorrhizal plants present an adaptive effect in arid climates [27]. Glomalin protein produced by arbuscular mycorrhizal fungi with a strong cementing capacity of soil particles [11, 28].

The working objectives of this study were testified by the large GRSP variations in both concentration and composition at different sites are mainly related to soil properties and mycorrhizal

colonization. The concentration of GRSP in mycorrhizal soils of *Pennisetum glaucum* seedlings varied from 1.08 mg g^{-1} to 3.01 mg g^{-1}. This result is in coincidence with that of Wright and Upadhyaya [11], who observed that the GRSP concentration was less than 2-3 mg g^{-1} in soil [29]. Am fungi inoculated pot soils maintained better soil structure, especially soil water-stable aggregates and GRSP, which are important for: increasing transportation of essential nutrients to host plant from soil and increasing stability against wind and water erosion (drought resistance) [4]. Auge *et al*., [30] reported that mycorrhizal soils had more water-stable aggregates and consequently higher soil moisture.

However, we are aware that more studies are needed for obtaining a better understanding of processes governing the stability of soil aggregates in our barren soils. We expect that AM fungi will be an important tool to face this challenge.

CONCLUSION

The present study showed that due to colonization by AM fungi, overall increase in the growth of pearl millet plant was observed. The significant growth of pearl millet plants may be attributed to mycorrhizal colonization as it is known to improve growth and provide other benefits to host plants in barren soil compositions. In this study, the AM inoculations has shown good correlations of the GRSP with the organic pool in the rhizosphere that had a positive influence add relative stability on soil aggregation.

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