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Effect of *Glomus* sp and *Gigaspora* sp. on *Vigna radiata* (L.) Under Water Stress Condition

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Abstract: A pot experiment was conducted to study the effect of arbuscular mycorrhizal fungi on green gram (*Vigna radiata* (L.) Wilczek) grown under water stress condition. Arbuscular mycorrhizal fungi (AMF) symbiosis can protect host plants against detrimental effects caused by drought stress. AMF were isolated and quantified in soil samples and morphological characters were studied. The isolated AMF were inoculated with Green gram seedlings and their physiological and bio chemical analysis were studied. The soil samples were collected from Courtallam (A) and Munnar (B). The soil samples A and B were analyzed for isolation and quantification of spores. The spores count was recorded as 18 and 22 spores/gm soil in samples soil A and soil B respectively. The highest counts of spores were present in maize field soil 79 spores/gm soil. The coconut field and banana field soil sample has moderate level of spores 54 and 45 spores/gm soil, respectively, while, the rice field soil sample containing onion root soil has 39 spores/gm soil. The presence of AM colonization in onion root is confirmed by microscopic studies. The percent root colonization was comparatively more in onion (91%) grown in maize field soil containing AM inoculation. The onion root soil sample was used for mycorrhizae morphological study. AM fungal spores were isolated from pot cultures containing onion root growth. The AM fungal species was identified only at genus level. The AM fungal species isolated from the present study sites belonging to two genera viz., *Glomus* sp and *Gigaspora* sp.

Key words: *Glomus* sp. · *Gigaspora* sp. · V*igna radiata* · Arbuscular mycorrhizal · Abiotic stress

INTRODUCTION

Abiotic stresses are caused by drought, salinity, high or low temperatures, light, deficient or excess nutrients, heavy metals, pollutants etc, either individually or in combination. The stress caused by abiotic factors alter plant metabolism leading to negative effect on growth, development and productivity of plants. If the stress become harsh and/or continues for longer period, it may lead to unbearable metabolic burden on cells leading to reduced growth and in extreme cases results in plant death [1]. Water deficit is an important abiotic factor limiting plant growth and yield in many areas. On the earth that is increasingly topical because of climate change and water shortage [2].

The symbiotic association between VAM fungi and root provides a significant contribution to plant nutrition and growth [3]. An Arbuscular Mycorrhizae (AM) fungus is a kind of soil fungi, to colonize the roots of most plants and form an organ called Arbuscular Mycorrhizae [4]. VAM 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. Effective nutrient acquisition by VAM-fungi is generally attributed to the extensive hyphal growth beyond the nutrient depletion zone surrounding the root and principal avoidance strategies of plants for adaptation to adverse soil strategies of plants for adaptation to adverse soil [5]. The beneficial effect of indigenous AM fungi on the nutrition of agricultural plants depends on both the abundance and type of fungi present in the soil. To identify the native AM fungi associated with green gram cultivated in different localities. *Glomus mosseae* was the most frequent mycorrhizal associate with Green gram [6].

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The improved water status and enhanced drought tolerance caused by VAM infection was due to absorption and translocation of water by external hyphae [7]. Arbuscular Mycorrhizal symbiosis is widely believed to protect host plants from detrimental effects of drought [8]. AM fungi interact synergistically with other microorganisms such as nitrogen fixing bacteria, phosphate solubilizing bacteria, biocontrol agents and plant growth promoting microorganisms to enhance plant growth and survival. AM fungi interact with these bacteria directly by providing niche and/or habitat or indirectly by modifying host physiology [9].

More and more experiments have indicated that AM was able to alter water relations and played great role in the growth of host plant in the conditions of drought stress [2]. In the last two decades, many reports on mycorrhiza were unfolding their importance in plant growth and development. With this background the present investigation was carried out on the effect of arbuscular mycorrhizal fungi on green gram (*Vigna radiata* (L.) Wilczek) grown under water stress condition.

MATERIALS AND METHODS

Soil Sample Collection: In the present study the soil samples were collected from Western Ghats (Courtallam) and Munnar field area. The plant roots were taken by loosening the soil around the root and then gently removed the plant material from the ground. Then it was transferred to sterile polythene bag and the sample was used for further analysis of isolation of mycorrhizae.

Isolation and Quantification of Spores: Soil samples were quantified by wet sieving and decanting method [10]. 250g of soil sample was mixed in 1000ml of water and allow heavier particles to settle down for few seconds. The liquid was poured through coarse - sieve (710 µm, 450µm, 75µm and 45 µm) to remove large pieces of organic matter. The filtrate was mixed with some more quantity of water, well shacked and allow heavier particles to settled for a few seconds. This suspension was passed through a sieve fine enough to retain the desired spores. The materials retained on the sieve to ensure that all colloidal material passed through the sieve. Small amounts of remaining debris were transferred to a Petri dish and examined under a dissecting microscope.

Spore Count: One gram of moist soil was added to 9 ml of distilled water in a test tube capped with a rubber stopper. The tube was then vigorously agitated by hand and 1 ml was immediately pipette in parallel lines onto a 9cm filter paper disc in a Petri dish. The filter paper was then scanned under a dissecting microscope and the spores were counted.

Carrier Based Inoculum Production: The isolated spores were used for inoculum production. Soil inoculum was considered to be more rapidly infective than spore inoculum. Four different field soil samples (Rice, Coconut, Maize and Banana) were used for the production of AM fungi. Onion (*Allium sepa*) was used as a host plant to propagate the AM fungal spores for 2 months.

Harvest and Storage: Entire pot culture was harvested by pruning plants till soil level, removing the compact soil mass from the pots and chopping the mycorrhizal roots. Before storage the soil mixture was air dried to the point at which there was no free water. After drying, the culture was packed in plastic bags, sealed to prevent further drying and stored at 5°C [11].

Root Sample Collection: Root sample of each plant species were collected from the pots. Roots samples with sand were carefully excavated intact, taking care that only roots definitely belonging to the same root systems of sampled species were collected. Root samples were collected from each pot and pooled in a single polythene box for a particular successional stage. Root samples were placed in a polythene bag and stored at 4°C until the laboratory processes. These roots were used for further identification [12].

Clearing and Staining Specimens: The roots were cut into pieces (0.5 cm to 1 cm) and heated in beaker containing 10% KOH for 20 minutes to 1 hour. KOH solution was cleared the host cytoplasm and nuclei and readily allows stain penetration. Pour off the KOH solution and rinse the root sample in a beaker with at least three complete changes of water. The root was covered in a beaker with alkaline H_2O_2 (NaOH - 3ml; H_2O_2 (10%) - 30ml; water- 567ml) and keep it at room temperature for 10 to 20 minutes. Rinse the root in beaker thoroughly with tap water to remove H_2O_2. The roots were covered in the beaker with 1% HCl and soak for 3 to 4 min and then pour off the solution. Do not rinse after this step because the specimens must be acidified for proper staining. Cover the root in the beaker with 0.01% Trypan blue - Lactic acid staining solution and warm it again in beaker for 10 min (Lactic acid solution: Lactic acid - 875 ml, glycerol - 63 ml, tap water - 63 ml: trypan blue - 0.1g). Excess of stain was removed and cover the root with above solution without trypan blue for destaining and mycorrhizal assay can be done [11].

Morphological Spore Characterization: The criteria for morphological spore characterization were mainly based on spore size and colour, wall structure and hyphal attachment to the roots was done according to Rosendahl [13].

Collection of Soil Sample: Soil sample were used for pot experiment must be sterilized. The sterilized soil (red soil, clay soil and sandy soil) was used for pot experiment to analyze the effect of AM on the physiological and biochemical changes on green gram plant. The soil sample was collected from in and around the Ayya Nadar Janaki Ammal College, Sivakasi.

Designing of Pot Experiment: Soil (red soil, clay soil and sandy soil) was sterilized by autoclaving at 15lbs pressure at 121°C for 15 minutes. The green gram seeds were surface sterilized. The sterilized soil was transferred to the pots (3/4). The infected root fragments and rhizosphere soils of the pots were used as inoculum and also for the preparation of seedbed. During preparation of seedbed, 3 treatments were applied, one with mycorrhizal inoculum, second with mycorrhizae water stress and third with only water stress. Then surface sterilized green gram seeds were spread onto the pots. After the intervals of 15, 30 and 45 days, effect of mycorrhizal inoculum were studied in the Green gram.

Design of the Experiment: AM1 - Arbuscular Mycorrhiza isolated from coconut field
AM2 - Arbuscular Mycorrhiza isolated from rice field
AM3 - Arbuscular Mycorrhiza isolated from maize field
AM4 - Arbuscular Mycorrhiza isolated from banana field
WAM1 - Water stress + Arbuscular Mycorrhiza isolated from coconut field
WAM2 - Water stress + Arbuscular Mycorrhiza isolated from rice field
WAM3 - Water stress + Arbuscular Mycorrhiza isolated from maize field
WAM4 - Water stress + Arbuscular Mycorrhiza isolated from banana field
S - Water stress only
C - Well watered control

Evaluation of Mycorrhizal Potential Morphological, Physiological and Biochemical Characterization: Effect of AM on the morphological, physiological and biochemical changes on green gram plant grown in sterilized soil sample were analyzed after 15, 30 and 45 days of seedling. After sowing of green gram, the day of emergence was noted for all the plants grown in soil and expressed in days. The percentage of root infection in all plants was calculated and expressed in percentage. The plants were uprooted carefully from the soil and washed with water and its length was measured in scale (cm). To measure the shoot length by from the starting point of the root to till the tip of the plant and also measure the root and shoot length of the control plant. The length of leaf was measured. Average length of the leaves was taken into consideration.

Effect of AM on Fresh Weight and Dry Weight of Green Gram Plant: The fresh weights of the crops were analyzed, the crops were uprooted from the soil and roots were washed with water. Then weigh the crops using balance. After drying, the crops are weighed for dry weight.

Effect of Am on Chlorophyll Content: The chlorophyll content of plant leaves were measured by the method of Arnon [14]. 100mg of leaf sample were ground with 80% acetone followed by centrifugation at 3000 rpm for 5 min. Absorbance of the supernatant was detected at 643 nm and 645 nm.

Chlorophyll a mg L^{-1} = 12.7 X A_{663} - 2.69 X A_{645}
X volume / 1000 X weight
Chlorophyll b mg L^{-1} = 22.9 X A_{645} - 2.69 X A_{664}
X volume / 1000 X weight
Total Chlorophyll mg L^{-1} = 20.2 X A_{645} + 8.02 X A_{663}
X volume / 1000 X weight

Determination of Carotenoids Content: The carotenoids content present in the acetone extract leaves were measured by using absorbance at 480nm, 638 nm and 645 nm.

Carotenoids content mg L^{-1} = A_{480}+(0.114 + A_{638}) X A_{645}

RESULT

In present investigation, AMF were isolated and quantified in soil samples and morphological characters were studied. The isolated AMF were inoculated with green gram seedlings and their physiological and bio chemical analysis were studied.

Isolation and Quantification of Spores from Soil Samples: In present study, the soil samples collected from Courtallam (labeled as A) and Munnar (labeled as B). The soil samples A and B were analyzed for isolation and quantification of spores. The spores count was recorded as 18 and 22 spores/gm soil in samples soil A and soil B respectively (Table 1).

Inoculum Production of Arbuscular Mycorrhizae and Quantification of Spores after Propagation in Onion: The total quantification of spores per gram soil was recorded in Table 2. The highest counts of spores were present in maize field soil 79 spores/gm soil. The coconut field and banana field soil sample has moderate level of spores 54 and 45 spores/gm soil respectively, while, the rice field soil sample containing onion root soil has 39 spores/gm soil.

Quantification of AM Infection in Roots of Onion: The presence of AM colonization in onion root was confirmed by microscopic studies. The percent root colonization was comparatively more in onion (91%) grown in maize field soil containing AM inoculation. Similarly, root colonization was observed in rice (75%), coconut (80%) and banana (63%) field sample containing onion root infected with AM fungi (Table 3).

Morphological Characterization: The onion root soil sample was used for mycorrhizae morphological study. AM fungal spores were isolated from pot cultures containing onion root growth. The AM fungal species was identified only at genus level. The AM fungal species isolated from the present study sites belonging to two genera viz., *Glomus* sp and *Gigaspora* sp. *Glomus* was the frequently associated mycorrhizae (Table 4).

Days of Emergence: In the present study, AM inoculated seedlings were germinated earlier than the water stressed AM inoculated seedlings. The control plants were germinated 4 days after inoculation. The day of emergence was 1 for AM1, AM4 and WAM2. The day of emergence was 2 for AM2, AM3, WAM1, WAM3 and stressed plants. WAM4 was germinated after 3 days of inoculation (Table 5).

Root Height: The length of the root was higher in AM1 infected green gram plant at 15, 30 and 45 days (3.6, 8.6 and 13.5cm respectively). The combination of water stress and AM infection has shown lesser impact on plant

Table 1: Isolation and quantification of spores in soil sample

S.No.	Soil sample	No. of spores/gm soil
1.	Soil A (Western Ghats)	18 ± 2.1
2.	Soil B (Munnar)	22 ± 2.2

Table 2: Quantification of spores after propagation in onion

S.No.	Soil sample	No. of spores/gm soil
1.	Coconut field	54 ± 2.2
2.	Rice field	39 ± 2.8
3.	Maize field	79 ± 4.2
4.	Banana field	45 ± 7.4

Table 3: Quantification of AM infection in root of onion

S.No.	Root sample	Percentage colonization (%)
1.	Coconut field	80
2.	Rice field	75
3.	Maize field	91
4.	Banana field	63

Table 4: Isolation of spores from soil sample

S.No.	Soil sample	Organism isolated
1.	Coconut field	*Glomus* sp
2.	Rice field	*Gigaspora* sp
3.	Maize field	*Glomus* sp
4.	Banana field	*Gigaspora* sp

Table 5: Effect of AM on days of emergence of green gram seedlings

S.No	Sample	Day of emergence (days)
1.	AM 1	1
2.	AM 2	2
3.	AM 3	2
4.	AM 4	1
5.	WAM 1	2
6.	WAM 2	1
7.	WAM 3	2
8.	WAM 4	3
9.	Stress only	2
10.	Control	1

Table 6: Effect of AM fungi and water stress on root height (cm) of green gram

		After planting of green gram at (cm)		
S.No	Sample	15 Days	30 Days	45 Days
1.	AM 1	3.6 ± 0.16	8.6 ± 0.17	13.5 ± 0.18
2.	AM 2	4.0 ± 0.10	7.5 ± 0.15	10.6 ± 0.15
3.	AM 3	6.4 ± 0.20	9.0 ± 0.20	13.5 ± 0.20
4.	AM 4	3.5 ± 0.20	5.8 ± 0.18	7.9 ± 0.20
5.	WAM 1	5.6 ± 0.18	7.1 ± 0.14	9.5 ± 0.25
6.	WAM 2	3.0 ± 0.09	9.5 ± 0.25	15.6 ± 0.20
7.	WAM 3	4.5 ± 0.10	11.1 ± 0.10	18.3 ± 0.10
8.	WAM 4	6.2 ± 0.20	9.2 ± 0.20	12.3 ± 0.10
9.	Stress only	3.8 ± 0.11	18.2 ± 0.17	22.2 ± 0.20
10.	Control	5.2 ± 0.17	9.2 ± 0.20	13.4 ± 0.10

height (Table 6). WAM3 plants recorded maximum height compared to water stressed plants alone (3.8, 18.2 and 22.2cm).

Shoot Height: AM infection was known to increase the shoot height by increasing nutrient uptake. In general, shoot height was higher for AM inoculated plant, when compared to water stressed AM inoculated plant. AM1 shoot height was 13.9, 27 and 40.5cm at 15, 30 and 45 days respectively, while, WAM1 plants recorded shoot height as 13.6, 26.4 and 39.4cm at 15, 30 and 45 days respectively. The water stress was declined the shoot height compared to control plants (Table 7).

Leaf Length: The length of the leaf was measured for AM infected, water stressed and control plants (Table 8). AM1 recorded higher leaf length 4.8, 5.6 and 6.1 cm at 15, 30 and 45 days respectively, while, WAM1 infected leaf length was 5.4, 4.7 and 4.5cm respectively and WAM3 infected leaf length 4.2, 5.5 and 6.7 respectively at 15, 30 and 45 days time interval.

Table 7: Effect of AM fungi and water stress on shoot height (cm) of green gram

		After planting of Green gram at (cm)		
S.No	Sample	15 Days	30 Days	45 Days
1.	AM 1	13.9 ± 0.09	27 ± 0.14	40.5 ± 0.20
2.	AM 2	14.6 ± 0.10	18.5 ± 0.12	22.5 ± 0.10
3.	AM 3	15.9 ± 0.20	25.5 ± 0.10	31.1 ± 0.15
4.	AM 4	19.0 ± 0.17	23.0 ± 0.20	27.2 ± 0.12
5.	WAM 1	13.6 ± 0.15	26.4 ± 0.17	39.5 ± 0.17
6.	WAM 2	14.0 ± 0.20	27.0 ± 0.09	31.5 ± 0.20
7.	WAM 3	13.4 ± 0.15	24.5 ± 0.20	35.4 ± 0.15
8.	WAM 4	13.5 ± 0.12	21.4 ± 0.10	29.5 ± 0.12
9.	Stress only	18.0 ± 0.17	28.5 ± 0.12	39.1 ± 0.20
10.	Control	12.9 ± 012	22.4 ± 0.15	32.6 ± 0.12

Table 8: Effect of AM fungi and water stress on leaf length (cm) of green gram

		After planting of green gram at (cm)		
S.No	Sample	15 Days	30 Days	45 Days
1.	AM 1	4.8 ± 0.16	5.6 ± 0.12	6.1 ± 0.12
2.	AM 2	4.1 ± 0.09	5.5 ± 0.10	6.4 ± 0.08
3.	AM 3	4.6 ± 0.05	4.8 ± 0.05	5.5 ± 0.15
4.	AM 4	2.5 ± 0.10	4.9 ± 0.10	6.5 ± 0.12
5.	WAM 1	5.4 ± 0.12	4.7 ± 0.05	4.9 ± 0.09
6.	WAM 2	3.6 ± 0.10	4.9 ± 0.15	5.6 ± 0.12
7.	WAM 3	4.2 ± 0.08	5.5 ± 0.09	6.7 ± 0.12
8.	WAM 4	4.4 ± 0.14	4.6 ± 0.12	4.9 ± 0.15
9.	Stress only	3.9 ± 0.09	5.5 ± 0.06	7.1 ± 0.09
10.	Control	4.5 ± 0.05	5.1 ± 0.08	6.2 ± 0.08

Table 9: Effect of AM fungi and water stress on fresh weight (gm) of green gram

		Fresh Weight (gm)		
S.No.	Sample	15 days	30 days	45 days
1.	AM 1	0.714 ± 0.01	1.070 ± 0.01	2.701 ± 0.02
2.	AM 2	0.453 ± 0.02	0.777 ± 0.02	1.088 ± 0.01
3.	AM 3	0.565 ± 0.04	1.048 ± 0.01	2.731 ± 0.01
4.	AM 4	0.347 ± 0.01	0.748 ± 0.03	1.701 ± 0.02
5.	WAM 1	0.771 ± 0.02	0.456 ± 0.04	1.061 ± 0.03
6.	WAM 2	0.347 ± 0.04	0.914 ± 0.05	1.605 ± 0.05
7.	WAM 3	0.548 ± 0.05	0.793 ± 0.01	1.506 ± 0.02
8.	WAM 4	0.458 ± 0.01	1.180 ± 0.02	2.210 ± 0.02
9.	Stress only	0.509 ± 0.02	1.322 ± 0.01	2.051 ± 0.03
10.	Control	0.473 ± 0.03	0.886 ± 0.05	1.211 ± 0.02

Table 10: Effect of AM fungi and water stress on dry weight (gm) of green gram

		Dry Weight (gm)		
S.No	Sample	15 Days	30 Days	45 Days
1.	AM 1	0.156 ± 0.02	0.245 ± 0.01	0.351 ± 0.02
2.	AM 2	0.086 ± 0.01	0.121 ± 0.03	0.311 ± 0.01
3.	AM 3	0.123 ± 0.04	0.178 ± 0.01	0.231 ± 0.02
4.	AM 4	0.075 ± 0.05	0.179 ± 0.03	0.235 ± 0.03
5.	WAM 1	0.201 ± 0.01	0.093 ± 0.02	0.321 ± 0.01
6.	WAM 2	0.056 ± 0.03	0.201 ± 0.01	0.411 ± 0.04
7.	WAM 3	0.146 ± 0.01	0.169 ± 0.02	0.251 ± 0.01
8.	WAM 4	0.125 ± 0.02	0.206 ± 0.04	0.301 ± 0.01
9.	Stress only	0.161 ± 0.02	0.349 ± 0.02	0.415 ± 0.02
10.	Control	0.096 ± 0.03	0.089 ± 0.03	0.126 ± 0.03

Fresh Weight and Dry Weight of Green Gram: Fresh weight and dry weight of green gram was recorded both under inoculated, non-inoculated and water stress condition. Mycorrhizal infested had significantly higher fresh weight and dry weight when compared to water stressed plants (Table 9 and 10).

Chlorophyll Content: The AM fungus enhanced the contents of Chl a, Chl b, Total chlorophyll content in all AM inoculated seedlings (Tables 11, 12 and 13). Chl a and Chl b content was higher in AM inoculated seedlings. WAM1 and WAM4 inoculated seedlings was also recorded higher chlorophyll content a and b. Total chlorophyll content was higher in water stressed AM inoculated seedlings compared to stressed plants (S). WAM1 has higher 16.36, 1959, 20.36 mg at 15, 30 and 45 days interval.

Carotenoids Content: Carotenoids content was observed in the Green gram crops. AM4 inoculated seedlings was synthesized higher carotenoid contend when compared to

Table 11: Effect of AM fungi and water stress on chlorophyll content a (mg)

S.No	Sample	Chlorophyll a (mg) 15 Days	30 Days	45 Days
1.	AM 1	9.65 ± 0.16	9.14 ± 0.14	9.75 ± 0.17
2.	AM 2	8.64 ± 0.14	9.02 ± 0.12	10.3 ± 0.17
3.	AM 3	8.77 ± 0.12	9.78 ± 0.16	10.8 ± 0.16
4.	AM 4	7.88 ± 0.09	11.4 ± 0.18	13.6 ± 0.18
5.	WAM 1	11.7 ± 0.18	15.9 ± 0.2	17.5 ± 0.19
6.	WAM 2	9.27 ± 0.15	9.28 ± 0.12	10.5 ± 0.15
7.	WAM 3	12.5 ± 0.18	12.1 ± 0.19	13.1 ± 0.18
8.	WAM 4	9.15 ± 0.15	13.1 ± 0.18	15.3 ± 0.2
9.	Stress only	9.91 ± 0.17	13.3 ± 0.18	14.2 ± 0.18
10.	Control	11.2 ± 0.18	12.8 ± 0.19	13.2 ± 0.18

Table 12: Effect of AM fungi and water stress on chlorophyll content b (mg)

S.No	Sample	Chlorophyll b (mg) 15 Days	30 Days	45 Days
1.	AM 1	14.2 ± 0.19	11.2 ± 0.18	15.2 ± 0.2
2.	AM 2	13.3 ± 0.18	12.8 ± 0.18	12.9 ± 0.23
3.	AM 3	13.7 ± 0.18	13.8 ± 0.17	14.2 ± 0.14
4.	AM 4	13.3 ± 017	16.0 ± 0.15	18.5 ± 0.18
5.	WAM 1	18.5 ± 0.19	22.2 ± 0.23	24.5 ± 0.25
6.	WAM 2	14.2 ± 0.12	17.4 ± 0.19	18.2 ± 0.12
7.	WAM 3	18.8 ± 0.14	15.3 ± 0.15	19.2 ± 0.16
8.	WAM 4	14.1 ± 0.12	16.9 ± 0.1	18.5 ± 0.15
9.	Stress only	14.2 ± 0.12	16.7 ± 0.2	18.6 ± 0.14
10.	Control	12.3 ± 0.15	16.7 ± 0.1	17.2 ± 0.16

Table 13: Effect of AM fungi and water stress on total chlorophyll content (mg)

S.No	Sample	Total chlorophyll content (mg) at 15 days	30 days	45 days
1.	AM 1	12.52 ± 0.17	9.89 ± 0.12	13.51±0.15
2.	AM 2	11.72 ± 0.15	11.31 ± 0.15	12.32±0.15
3.	AM 3	12.21 ± 0.17	12.12 ± 0.15	13.54±0.15
4.	AM 4	11.72 ± 0.15	14.14 ± 0.17	16.42±0.19
5.	WAM 1	16.36 ± 0.19	19.59 ± 0.19	20.36±0.19
6.	WAM 2	12.52 ± 0.17	15.35 ± 0.18	17.32 ± 0.2
7.	WAM 3	16.57 ± 0.19	13.54 ± 0.15	16.21±0.19
8.	WAM 4	12.32 ± 0.17	14.95 ± 0.18	16.35±0.19
9.	Stress only	12.53 ± 0.17	14.75 ± 0.18	16.45±0.19
10.	Control	14.34 ± 0.18	14.55 ± 0.18	15.56±0.18

Table 14: Effect of AM fungi and water stress on carotenoid content (mg)

S.No	Sample	Carotenoid content (mg) at 15 days	30 days	45 days
1.	AM 1	0.911 ± 0.15	1.267 ± 0.17	2.362 ± 0.2
2.	AM 2	0.766 ± 0.14	1.069 ± 0.17	2.254 ± 0.2
3.	AM 3	0.810 ± 0.15	1.028 ± 0.17	2.321 ± 0.2
4.	AM 4	0.772 ± 0.14	1.171 ± 0.16	2.553±0.21
5.	WAM 1	1.237 ± 0.17	1.134 ± 0.16	2.213 ± 0.2
6.	WAM 2	0.941 ± 0.15	0.989 ± 0.15	1.254±0.19
7.	WAM 3	1.266 ± 0.17	1.005 ± 0.16	1.658±0.19
8.	WAM 4	0.768 ± 0.14	1.152 ± 0.17	2.314 ± 0.2
9.	Stress only	0.905 ± 0.15	1.143 ± 0.17	2.014 ± 0.2
10.	Control	0.964 ± 0.15	1.100 ± 0.16	2.031 ± 0.2

AM1, 2 and 3. WAM4 inoculated seedlings were produced higher carotenoids content (0.768, 1.152 and 2.314 mg at 15, 30 and 45 days interval). Stress only and control crops were produced maximum carotenoids content. Mycorrhizal plants were synthesized higher carotenoids content when compared to non-mycorrhizal plants (Table 14).

DISCUSSION

The soil samples A and B were analyzed for isolation and quantification of spores. The spore count was recorded as 18 and 22 spores/gm soil in samples soil A and soil B respectively. Spore density in the dried soil was similar to that in the control soil under drought conditions. The strategy of the AM fungal community consisted of reducing external hyphae but moderating the reduction in arbuscular and maintaining a similar proportion of vesicles in roots and spores in soil [15]. The percent root colonization was comparatively more in onion (91%) grown in maize field soil containing AM inoculation. Similarly, root colonization was observed in rice (75%), coconut (80%) and banana (63%) field sample containing onion root infected with AM fungi. Similarly, inoculating *Senna spectabilis* with VAM resulted into a 67.8%, no VAM contamination as evident in control which showed 0% colonization [16].

The AM fungal species isolated from the study sites belonging to two genera, *Glomus* sp and *Gigaspora* sp. *Glomus* was the frequently associated mycorrhizae. The AM fungal spores in the rhizosphere of Green gram were identified as to three genera: *Glomus*, *Gigaspora* and *Scutellospora* and *Glomus mosseae* was the most frequent mycorrhizal associate. Native AM fungi in green gram showed low species diversity [6].

In the present study, AM inoculated seedlings were germinated earlier than the water stressed AM inoculated seedlings. The length of the root was higher in AM1 infected green gram plant at 15, 30 and 45 days (3.6, 8.6 and 13.5cm, respectively). In general, shoot height was higher in AM inoculated plant, when compared to water stressed AM inoculated plant. Mycorrhizal infested plant showed significantly higher fresh weight and dry weight when compared to water stressed plants. The symbiosis between an AM fungal community and *L. tenuis* plants was affected by a 35 days drought period resulting in reduced total root length, root length colonized, arbuscular colonization and number of entry points per

unit of colonized root length although drought treatment did not affect the vesicle colonization [15]. The enhanced height increment in *Senna spectabillis* could be attributed to the VAM colonization. Mycorrhizal infection has known to enhance plant growth by increasing nutrient uptake, the uptake of nitrogen, phosphorus and potassium and also greater rates of photosynthesis. Difference in height augmentation in inoculated and non-inoculated plants was significant, although the height increase in inoculated plant was higher. This could be due to the "lag phase" in effect of inoculated mycorrhiza [16]. In present study, Water stressed AM infected green gram was higher root height. The length of the root was higher in AM1 infected with green gram at 15, 30 and 45 days (3.6, 8.6 and 13.5cm). Green gram infected with AM3 was higher root height (6.4, 9.0 and 13.5cm). Inoculated plants had higher number of roots than non inoculated ones, though the increment was not significant at 5% level. Mycorrhizal inoculation is known to enhance the plants absorption of more nutrients especially phosphorus via an increase in the absorbing surface area. Higher plant growth rate was enhanced a more roots for plant growth [16].

The AM fungus enhanced the contents of Chl a, Chl b, Total chlorophyll content in all AM inoculated seedlings. AM4 inoculated seedlings were synthesized higher carotenoids content when compared to AM1, 2 and 3. Mycorrhizal infested plants were synthesized higher carotenoids content when compared to non-mycorrhizal plants. VAM fungi significantly enhance the net photosynthesis by increasing total chlorophyll and carotenoids contents ultimately increasing carbohydrate accumulation. Photosynthesis and transpiration rates of mycorrhizal *Satsuma mandarian* trees are higher than non mycorrhizal trees. Mycorrhizal turf, creeping bent grass has maintained significantly higher chlorophyll concentration than non mycorrhizal turf during drought period [3].

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