

Influence of Arbuscular Mycorrhizal Fungi on The Growth of Green Gram (*Vigna radiata* L.) Grown Under Water Stress Conditions

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Abstract: The Arbuscular Mycorrhizal Fungi (AMF) is well known to increasing the shoot length of plant species by transferring the insoluble soil nutrients. Relative Water Content (RWC) was higher in all mycorrhizal infected plants than non-mycorrhizal plants (22%). Under water stress condition WAM1 (37%), WAM2 (41%), WAM3 (44%) and WAM4 (28%) recorded maximum level of RWC. The total soluble sugars in the leaves of all Green gram plant showed a decrease in mycorrhizal seedlings than water stressed mycorrhizal seedlings. The starch content was higher in seedling inoculated with mycorrhizal crops when compared to water stressed mycorrhizal crops. The effect of AM inoculation in the protein content showed a greater increase in mycorrhizal plants than in control seedlings and water stressed plants. At the same time, proline content had recorded very low values in AM inoculated seedlings. SDS PAGE study revealed that, there was a prominent protein expression pattern in AM infected soil samples compared to non-infected soil samples. In AAS analysis, the soil inoculated with AM fungi produced high level of calcium, ferrous and potassium content while compared to non AM inoculated soil sample.

Key words: VAM • Greengram • *Vigna radiata* • AASpectrophotometer • SDS-PAGE

INTRODUCTION

Vesicular arbuscular mycorrhizal (VAM) fungi is (Zygomycetous fungi from the order Glomales) the most common symbiosis of the plant kingdom and colonize more than 80% of vascular plants [1]. A symbiosis refers to an association of living organisms that benefits both partners, enabling them to survive and reproduce more successfully and also it acquires increased resistance to environmental stresses such as drought, cold and root pathogens [2]. There are extensive microbial activities in rhizosphere soil which is colonized by a wide range of microbes having important effects on plant nutrition, growth and health. Generally VAM fungi are known to enhance phosphate uptake, which in turn improve plant growth and nitrogen fixation. VA-mycorrhizae are associated with the roots of angiosperms, gymnosperms, pteridophytes and bryophytes and are important in agriculture, horticulture and forestry and also it protected some phytopathogenic infection [3]. AM fungi assist the plants to absorb mineral nutrients from the soil

predominantly low existing elements like molybdenum (Mo), cobalt (Co) and phosphorus (P) etc. These fungi are always stimulate absorption of zinc (Zn) and copper (Cu) and also increase plant resistance to various stresses like drought, salt, heavy metal and water [4]. However, AM fungi have been interact with some another beneficiary soil microorganisms like N₂ fixer, phosphate solubilizer and PGPS producing microbial strains [5]. AM fungal strains are used to enhance production of roots, shoots and fruits and it is used as a substitute biofertilizer. Generally it is used to maintain the soil nutrients and water resources for next cohort of cultivated area. More and more reports are coming on the ability of AM fungi's ability to alter water relations and play a great role in the growth of host plant in the conditions of drought stress [6]. Considering the importance of AM fungal groups as a alternative biofertilizer for the efficacy of agriculture environment. Hence the present study was undertaken to investigate on influence of Arbuscular Mycorrhizal fungi on the growth of *Vigna radiata* (L.) (Green gram) grown under water stress conditions.

MATERIALS AND METHODS

Pot experiment was carried out to study the influence of mycorrhizal associations on the growth of green gram under water stress conditions.

Collection of Root and Rhizospheric Soil Sample and Identification of Mycorrhizal Strains: In the present study, the rhizosphere soil and root samples were collected from Western Ghats of Tamilnadu. The plant roots were dug out and then the rhizospheric soil samples were carefully taken by sterile air tight polythin cover and transferred to the laboratory.

Measurement of Relative Water Content: Relative water content was measured by leaf disc method [7]. In brief, totally 25 discs (1cm) of each sample were taken with closed punch. Fresh weight of the sample was recorded by using electronic balance. The discs were floated in petriplates containing distilled water at room temperature for 4 hours. Turgid weights of the discs were recorded after carefully blotting the excess water. Discs were dried and their dry weight were then recorded.

Biochemical Estimation

Estimation of IAA, Carbohydrates, Reducing Sugars and Starch: The Indole Acetic Acid was quantitatively analysed by the method of Gotdan and Webber [8]. Quantitative estimation of total carbohydrates and reducing sugars were carried out according to Hodge and Horfrier [9]. Estimation of starch was performed by Anthrone method [10].

Estimation of Protein and Aminoacid: The stress proteins were extracted and then estimated according to the method of Lowry *et al.* [11]. The estimation of amino acid was carried out by Bieleski and Turner [12].

Estimation of Potassium and Phosphorus: Nine leaf samples for each treatment were collected from the sixth nodes of the shoots was dried at 65°C for 48 hours. Total leaf N content was determined using the Kjeldahl method. Phosphorus estimation was done by the vanatademolybdate yellow colorimetric method using a Spectrophotometer and also K by atomic absorption method [13]. Total sucrose content of test leaf sample was determined according to a modified Anthrone method [14].

Estimation of Ca, Fe and K: The uninoculated and inoculated soil samples were digested with concentrated nitric acid (HNO₃) and 30% hydrogen peroxide and then micro nutrients such as Ca, Fe and K were determined by an Atomic Absorption Spectrophotometer.

Measurement of Soil pH: The soil pH was measured by using pH meter. 1 gm of soil sample from mycorrhizal, water stressed mycorrhizal, stress only, control crops and sterilized soil were collected in a series of test tubes. About 5 ml of distilled water was added and mixed well. Then pH was measured by using pH meter.

SDS PAGE- Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis: SDS-PAGE (12%) was performed according to the method of Laemmli [15] under reducing conditions. The molecular weight was determined by interpolation from a linear semi-logarithmic plot of relative molecular weight versus the Rf value (relative mobility) using a standard molecular weight marker.

RESULTS

In this present study, the isolated AMF were inoculated with Green gram seedlings and their physiological and bio chemical parameters were studied.

Relative Water Content (RWC): The main effect of AM fungi, level and the interaction of fungi and moisture were significant. RWC was higher in all mycorrhizal infected plants (44%) than non-mycorrhizal plants (22%). Under water stress condition WAM1 (37%), WAM2 (41%), WAM3 (44%) and WAM4 (28%) recorded maximum level of RWC (Table 1).

Analysis of soil pH After 45 days of Incubation: In the present study, the pH was varied and ranged between 8.47 and 8.81. Mycorrhizal inoculated soil samples were recorded lower pH (8.68 to 8.73) over the the control (8.8) (Table 2).

Biochemical Parameters

Estimation of IAA: In the present study, the leaf IAA content of all mycorrhizal inoculated seedlings had significantly showed a greater value than the non-mycorrhizal seedlings. Whereas, the WAM4 and S plants recorded as 0.05, 0.09, 0.15 mg/gm and 0.03, 0.07, 0.14 mg/gm at 15, 30 and 45 days intervals respectively.

Table 1: Effect of AM fungi and water Stress on Relative water content (RWC) and Water saturation deficit (WSD)

S.No.	Sample	Relative water content (RWC) (%)	Water saturation deficit (WSD)
1.	AM 1	24.30	75.70
2.	AM 2	38.46	61.54
3.	AM 3	44.23	55.77
4.	AM 4	45.76	54.24
5.	WAM 1	37.07	62.93
6.	WAM 2	41.03	58.97
7.	WAM 3	44.08	55.92
8.	WAM 4	27.86	72.14
9.	Stress only	22.02	57.98
10.	control	29.80	70.20

Table 2: Analysis of soil pH after 45 days of incubation.

S.No	Soil Sample	pH
1.	Sterile Soil	8.47
2.	AM 1	8.73
3.	AM 2	8.69
4.	AM 3	8.71
5.	AM 4	8.68
6.	WAM 1	8.74
7.	WAM 2	8.76
8.	WAM 3	8.70
9.	WAM 4	8.69
10.	Stress only	8.81
11.	Control	8.80

WAM2 and WAM3 were recorded 0.05, 0.08, 0.14 mg/gm and 0.03, 0.08, 0.14 mg/gm at 15, 30 and 45 days interval respectively (Fig. 1).

Estimation of Total Soluble Sugars: The total soluble sugars in the leaves of all Green gram showed a decrease in mycorrhizal seedlings than water stressed mycorrhizal seedlings. The total soluble sugar content was recorded as 0.08, 0.47 and 1.80 mg/g for WAM3 inoculated seedlings at 15, 30 and 45 days intervals. WAM4 was recorded as 0.34, 0.52 and 1.60 mg/gm. AM2 was recorded as 0.14, 0.72 and 1.56 mg/gm sugar content at 15, 30 and 45 days (Fig. 2).

Estimation of Starch: The maximum starch content was recorded in seedling inoculated with mycorrhizal crops. Amongst, the AM1 inoculated seedling had showed highest content (0.09, 0.54 and 0.92 mg/gm) at 15, 30 and 45 days intervals. WAM1 inoculated seedlings was synthesized 0.09, 0.42, 0.85 mg/gm at 15, 30 and 45 days intervals. The stress only and control crops produced minimum starch content (Fig. 3).

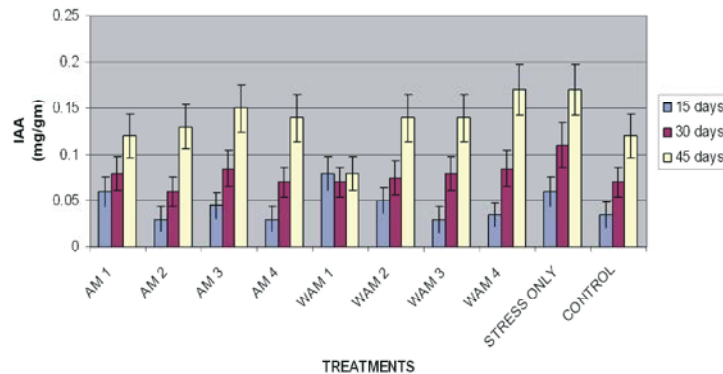


Fig. 1: Estimation of Auxins (Indole- 3 -acetic acid) (mg/g).

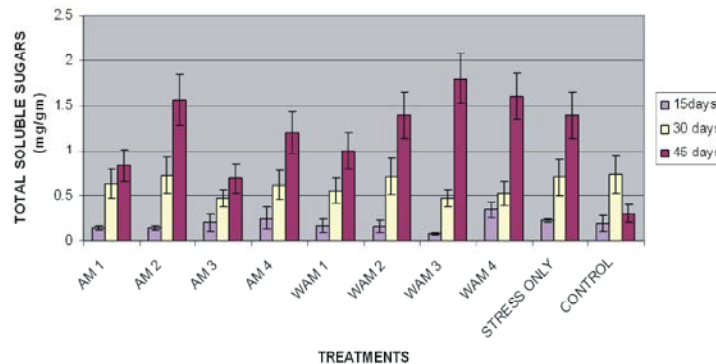


Fig. 2: Estimation of Total Soluble Sugars (mg/g) in Green gram.

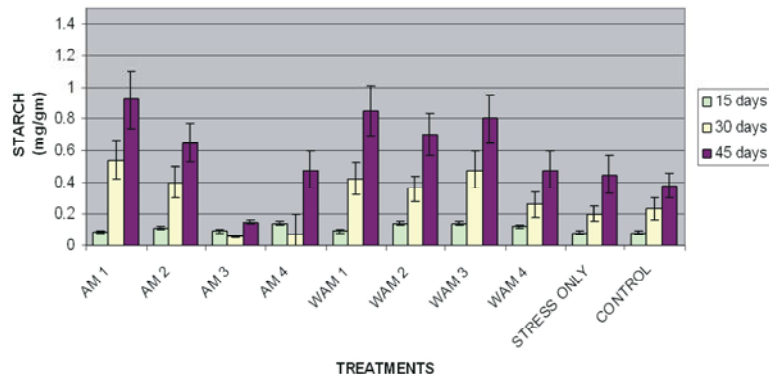


Fig. 3: Estimation of Starch (mg/g) in Green gram.

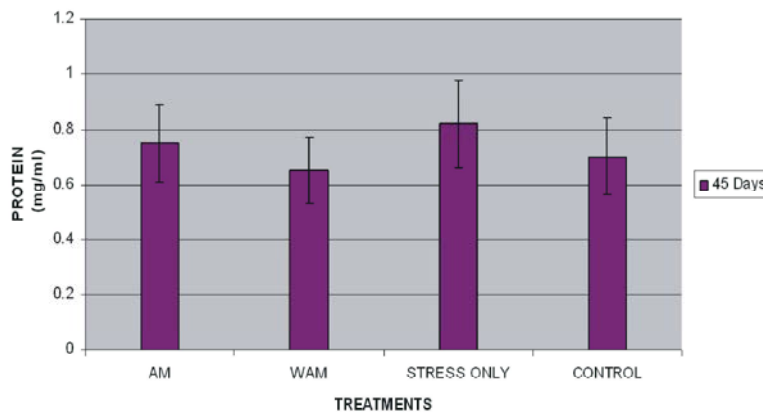


Fig. 4a: Estimation of Protein (mg/g) by UV- Absorption method.

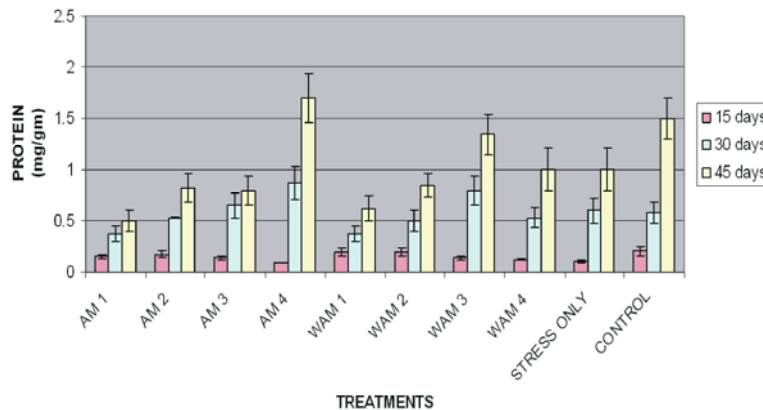


Fig. 4b: Estimation of Protein (mg/g) in Green gram.

Estimation of Protein: The effect of AM inoculation in the protein content showed a greater increase in mycorrhizal plants than in control seedlings and water stressed plants (Fig. 4a). The protein content was higher for AM4 inoculated seedlings 0.87 and 1.70 mg/gm at 30 and 45 days intervals. The WAM3 inoculated seedlings recorded 0.14, 0.80 and 1.34 mg/gm at 15, 30 and 45 days intervals (Fig. 4b).

Estimation of Proline: In the present study, the leaf proline content recorded very low values in AM inoculated seedlings. WAM1 recorded 0.74, 0.93 and 1.90 mg/gm at 15, 30 and 45 days interval, while AM2 inoculated seedlings produced 0.41, 0.67 and 1.00 mg/gm proline at 15, 30 and 45 days intervals (Fig. 5).

SDS PAGE-Analysis: Our current SDS PAGE study revealed that, there was a prominent protein expression

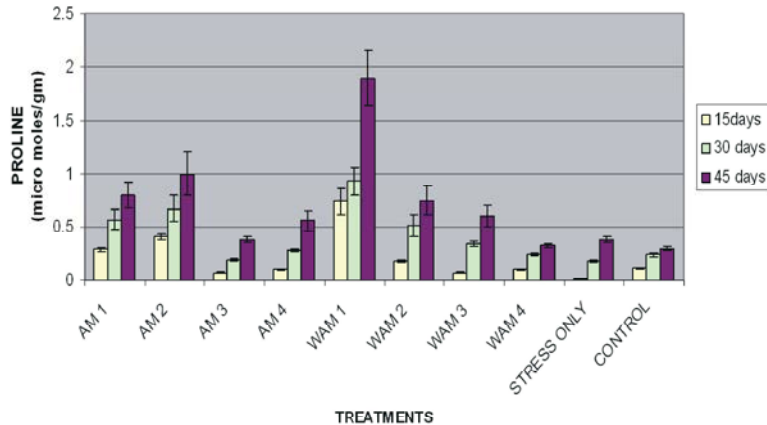


Fig. 5: Estimation of Proline (μ moles/g) in Green gram.

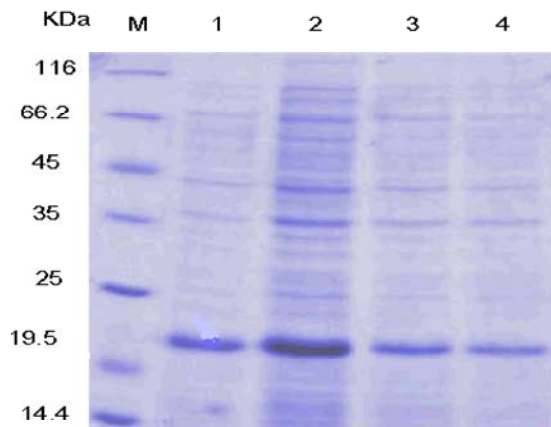


Plate 1: SDS PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis). M - Marker; 1 - AM inoculated; 2 - Water stress+ AM inoculated; 3 - Stress only and 4 - control.

Table 3: Estimation of Calcium, Ferrous and Potassium by AAS Analysis

S.No.	Sample	Calcium (ppm)	Ferrous (ppm)	Potassium (ppm)
1.	Before AM inoculation	1.421	0.311	7.682
2.	After AM inoculation	1.589	0.6663	12.42

pattern found in AM infected leaf samples compared to non-infected leaf samples. We were able to spot specific band pattern in AM infected plants compared to control and stressed plants (Plate 1).

AAS Analysis: In AAS (Atomic Absorption Spectroscopy) analysis, soil inoculated with AM fungi contained more amount of metal content while compared to uninoculated soil samples. Before inoculation of AM fungi, the Calcium, Iron and Potassium were present as

1.421, 0.311 and 7.682ppm. After inoculation of AM fungi, the content of Calcium, Iron and Potassium was 1.589, 0.6663 and 12.42ppm respectively (Table 3).

DISCUSSION

Soil provides the physical support for the root system and as the reservoir of water and nutrients, which are essential for plant growth [16]. RWC was higher in all mycorrhizal infected plants than non-mycorrhizal plants (22%). Under water stress condition WAM1 (37%), WAM2 (41%), WAM3 (44%) and WAM4 (28%) recorded maximum level of RWC. Leaf RWC at both plant growth stage decreased with increase in water stress and RWC was higher in all mycorrhizal than non mycorrhizal plants irrespective of soil moisture level.

In the present study, Mycorrhizal inoculated soil samples recorded lower pH when compared to control (8.8). pH of the mycorrhizal inoculated soil was between 8.68 and 8.73 pH. In consistence with this present study, arbuscular mycorrhizal fungi vary in their tolerance to pH and sporulation in certain species decrease with increase in pH. But no significant correlation was observed between spore count and pH [17]. The high level of metal content was recorded in AAS analysis while compared to uninoculated soil samples. Before inoculation of AM fungi, the calcium, ferrous and potassium was 1.421, 0.311 and 7.682ppm. This is correlated with the earlier report of Manoharan *et al.* [18] who reported that the inoculation of AM fungi as found to be increased the content of calcium, ferrous and potassium was 1.589, 0.6663 and 12.42ppm respectively.

Auxins are growth regulators which are essential for plant growth. In the present study, the leaf IAA content of all mycorrhizal inoculated seedlings showed a greater

amount than the non-mycorrhizal seedlings. From this results it can be conclude that the bacterial cultures had influence to produce the IAA at high level in pot experiments [19]. The total soluble sugars in the leaves of all Green gram in the present study showed a decrease in mycorrhizal seedlings than water stressed mycorrhizal seedlings. The starch content was higher in seedling inoculated with mycorrhizal crops when compared to water stressed mycorrhizal crops. The decrease in the soluble sugars and soluble starch in the leaves may be due to the fact that the VAM fungi utilize 10-20% of net photosynthate in exchange for the transfer of nutrients to the host to lead a symbiotic life. The result obtained was in accordance with previous report of Manoharan *et al.* [18]. Who reported that the decrease in soluble sugars and soluble starch may be due to the translocation of carbohydrate produced by the host for the fungal partner. The effect of AM inoculation in the protein content showed a greater quantity in mycorrhizal inoculated plants than in control seedlings and water stressed plants. The VAM fungus had influenced the level of total protein, amino acid and nitrogen fractions in host plants and induced the plant growth [18].

Proline is an important amino acid in plant under drought stress that prevents oxidation from the inside of the cells. Also it regularizes osmotic pressure of plant under drought stress for absorbing water. Therefore proline accumulation rate increased in Coriander under drought stress. Water deficits induce dramatic increase in the proline content at root apex in water stressed plants [20]. In the present study, the leaf proline content recorded very low values in AM inoculated seedlings. WAM1 recorded 0.74, 0.93 and 1.90 mg/gm at 15, 30 and 45 days interval, while AM2 inoculated seedlings produced 0.41, 0.67 and 1.00 mg/gm proline at 15, 30 and 45 days intervals. This observation is in accordance with the earlier report of Manoharan *et al.* [18].

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