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ORIGINAL ARTICLE

Effects of *Glomus mosseae* and *Azospirillum* on the growth behavior of Black gram *Vigna mungo* (L.) Hepper

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ABSTRACT

Azospirillum lives in natural soil or in close associations with plants in the rhizosphere. It is helpful to plants and important to farmers because it is able to fix nitrogen (N_2). It can convert nitrogen gas in the air into nitrogen bound up in amino acids and proteins. Arbuscular Mycorrhizal Fungi (AMF) can increase the capability of the root systems to absorb and translocation of Phosphorus (P) and minor elements through an extensive network of mycelium. AMF are commonly associated with legumes and can increase nutrient uptake of plants growing in high phosphate fixing soils. The present study the efforts of single and duel inoculation of Glomus mosseae and Azospirillum on black gram Vigna mungo (L.) Hepper. The AMF Glomus mosseae + Azospirillum inoculation plants resulted in production of highest biomass such as plant, shoot length, root length, number of leaves, number of root nodules, fresh and dry weight of black gram plants and the biochemical content such as total chlorophyll, total sugar and protein. The nutrient contents such (macro and micro nutrients). The inoculation of Glomus mosseae + Azospirillum showed an enhanced among the parameters when compared to control seedlings.

Key words: *Glomus mosseae, Azospirillum, Phosphorus, Nitrogen, AMF, Morphological and Biochemical parameters.*

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INTRODUCTION

Biofertilizer is defined as a substance which contains living microorganisms and is known to help with expansion of the root system and better seed germination. The microorganisms containing biofertilizers can be the tools we could change apply of chemical fertilizers. Biofertilizers are products containing living cells of different types of microorganism, which have an ability to convert nutritionally important elements to available form through biological processes. In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies [1]. There is a great interest in establishing novel associations between higher plants and various N_2-fixing microorganisms and Phosphorus solubilizing fungi [2].

Azospirillum are gram-negative to gram-variable, curved-rod shape, motile, oxidase positive and exhibit acetylene-reduction activity (ARA) under micro-aerophilic conditions. *Azospirillum* spp. has been identified mainly as rhizosphere bacteria and its colonization of the rhizosphere has been studied extensively along with reporter gene fusion [3]. *Azospirillum* spp. is a most studied plant growth promoting bacteria because of its ability to colonize in roots of different plant species and most of the plants are of agricultural importance [4]. These bacteria use atmospheric nitrogen for the synthesis of the cellular proteins. Cellular protein is mineralized after the death of the cell, thus contributing to the availability of nitrogen for wild plants and crops [5].

AMF, are particularly ubiquitous in soil and create symbiotic associations with most terrestrial plants including agricultural crops, cereals, vegetables, and horticultural plants. In agriculture, several factors such as host crop dependency to mycorrhizal colonization, tillage system, fertilizer application, and the potential of AMF inoculam, affect plant response and plant benefits from mycorrhizae [6]. Interest in AMF propagation for sustainable agriculture is increasing due to its role in the promotion of plant health, and improvements in soil fertility and soil aggregate stability. These fungi can be utilized effectively for increasing yields while minimizing use of pesticides and inorganic fertilizers [7].

N_2 is an integral component of many compounds including chlorophyll and enzymes essential for plant growth processes. It is essential component of amino acid and related proteins. N_2 is essential for carbohydrate use within plants and stimulates root growth and development as well as the uptake of other nutrients. This element encourages above ground vegetative growth and gives a deep green colour to the. It is recognized that nitrogen is one of the key elements of soil fertility leaves [8]. Blackgram being a leguminous crop is capable to fix atmospheric N_2 through symbiosis.

Phosphorus (P) is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and transfer of genetic characteristics from one generation to the next. Promotes early root formation and growth, Improves quality of fruits, vegetables, and grains, Increases water-use efficiency.

Black gram is the third important pulse crop in India. It is annual pulse crop and native to central Asia. It is also extensively grown in West Indies, Japan and other tropics and subtropical countries. The seeds are highly nutritious containing higher amount of protein (24-26 %) and rich in potassium, phosphorus and calcium with good amount of sodium. It is also reported to be rich in vitamin A, B_1, $B_{3,}$ mineral and vitamins. It has some medicinal properties, like curing diabetes, sexual dysfunction, nervous disorder, hair disorders, digestive system disorders and rheumatic afflictions [9].

MATERIALS AND METHODS

Seed

The seeds black gram *Vigna mungo* (L.) Hepper varieties (IPU-941) were obtained from Local Agro seeds centre Dharmapuri Taluk and Dharmapuri district, Tamil Nadu, India. The seeds uniform size, colour and weights were chosen for experimental purpose. Seeds were surface sterilized with 0.1 percent mercuric chloride solution and washed thoroughly with tap water and then with distilled water.

AMF and *Azospirillum* Collection and Inoculation;

The AMF and *Azospirillum* were collected at District Forest Office (Modern Nursery Division) the AMF species employed was *Glomus mosseae*. The inoculum used consisted of Vermiculite soil containing spores (800 to 1000 / 200g dry soil), hyphal fragments and fine roots of maize infected with *Glomus mosseae. Azospirillum* was applied @ 1 kg ha^{-1} by mixing with seed.

Pot culture experiments

Twenty five seeds of black gram were sown in each earthen pots separately were filled with 5 kg/pot of sandy and Red loam soil. The experiment was arranged as a randomized black design with five replicates for each treatment. The following replicates were carried out with the following treatments. T_1- Control, $T_{2\,-}$ AM fungi, (*G.mosseae*) T_3 – *Aszospirillum* and T_4- *G.mosseae* + *Azsopirillum*

Black gram plants were collected at 50^{th} days and their shoot and root length was measured and recorded. The roots were dipped in water to remove adhering soil particles and washed with tap water and distilled water the number of root nodules were estimated. Dry weights of the plants were determined after drying at even 80°C of a constant weight. The chlorophyll content was assayed according to [10] the extraction was made from a 100 mg fresh sample in 25 mL acetone (80%) in the dark at the room temperature and measured at 470, 646 and 663 nm with a UV sepectrophotometers. The sugar and protein content of plant leaf materials were estimated according to [11] and [12]. Nitrogen (N) content was extracted from sulfuric acid using the semi micro kjeldhal method [13]. Phosphorus (P) was extracted by nitric acid and perchloric acid digestion and measured using the vanadoso-molybdophoshoric colorimetric method [14]. Potassium (K) was assayed using a flame spectrophotometer [16] calcium [17], Magnesium [15], Zinc, copper, Sulphur, iron and manganese [18].

RESULTS AND DISCUSSION

Biofertilizers will be the best solution to replace chemical fertilizers. Biofertilizers are the carrier-based arrangements containing mostly valuable strains of microorganisms in adequate number, which are useful for N_2 fixation. Amongst the nutrients, nitrogen is the only nutrient, which play major role in synthesis of chlorophyll, amino acids and protein building blocks [19].

The pot experiment was carried out to inoculation with *G. mosseae* and *Azospirillum* on morphological and biochemical contents were presented in Tables 1. The experimental results, maximum values of root length (29.8cm), shoot length (42.7cm), No of leaves (47.4), total leaf area (37.2), number of root nodules (81.5), fresh weight (48.52 g), Plant dry weight (23.64g) total chlorophyll (4.6 mg.fr.wt), total sugars (7.6) and protein (18.28 mg.fr.wt) was recorded in AMF + *Azospirillum* application at 50 DAS of black gram plant. The maximum root length (22.5cm), shoot length (34.2cm), No of leaves (38.2), total leaf area (29.7), number of root nodules (51.2), fresh weight (40.48 g), Plant dry weight (18.43g) total chlorophyll (3.22 mg.fr.wt), total sugars (3.39) and protein (9.5 mg.fr.wt) was recorded in control treatments at 50 DAS of black gram plants.

The data indicated that the dependence of black gram plant growth on inoculation with *G. mosseae* + *Azospirillum* the increased the root and shoot length on all sampling days. The increase in plant height might be attributed to the N2-fixation by *Azospirillum* which inturn make the essential nutrients available to the plant growth and development. These substances have also been reported to increase the activity of cell division and cell elongation ultimately leading to an increased plant height. Similar results have also been reported from Fallik and Okon [20] in *Setaria italica*, cauliflower as Jawar moti [21]. Arbuscular Mycorrhizal Fungi (AMF) symbioses have been shown to benefit growth of many field crops in large part due to the extensive hyphal network development in soil, more efficient exploitation of nutrients, and enhanced plant uptake [22]. The AMF infection is recognized to augment plant growth by increasing nutrient uptake. The higher height increment registered with duel inoculated plants could be as a result of enhanced both organic and inorganic nutrient absorption and greater rates of photosynthesis which obviously could have give to an increase in plant growth [23].

The *G. mosseae* + *Azosprillum* inoculated plants were increased in the fresh and dry weights of black gram plants. This result was in correspond to [24] who reported that inoculation with the mycorrhizal fungus *Glomus fasciculatum* enhanced peanut growth and increased its dry matter more than 2-fold compared with control. AMF inoculated plants have the fungal hypha increase root surface area, resulting in exploring higher volume of soil and overcoming the water and nutrient depletion zones around the roots leading to increased water and nutrient content [25]. Inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, plant height, number of leaves, leaf area and seedlings length [26].

The pigment content of total chlorophylls and biochemical content such as total sugars and protein content was significantly increased the duel inoculation of *G.mosseae +Azsopirillum.* The effect of N_2 fixing and phosphate solubulizer microorganisms exhibited significant differences in total chlorophyll content. Among the treatments, the total chlorophyll content was significantly higher in the treatment AMF + *Azospirillum* at 50th days of black gram plants. This could be due to the beneficial effect of duel inoculants which helped the plants to get more nitrogen which is the component of chlorophyll molecule. The delayed leaf senescence has also been attributed to higher total chlorophyll content [27].and Free-living nitrogen-fixing bacteria eg *Azotobacter chroococcum* and *Azospirillum lipoferum*, were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to Gibberellic Acid (GA) and Indole Acetic Acid (IAA), which could stimulate plant growth, and photosynthesis [28].

Sugar is an important energy constituent needed for all living organisms. The concentrations of soluble sugar indicate the physiological activity of plant organisms. The total sugar content was higher in combined inoculation of microorganisms similarly the control plants have lowest sugar content. Increased accumulations of sugars in black gram plant is due to G. *mosseae* + *Azsopirillum* application is in conformity with the earlier studies in different micro organisms on crop plants such as maize [29] and , *Albizia lebbek* [30], Suresh and Bagyaraj [31] reported that AM inoculation increased the quantities of sugars and amino acids in plant tissue.

Protein is one of the reserve food materials which are utilized for the growth of seedlings. The highest protein content of black gram plant was recorded in the treatment of G. *mosseae* + *Azsopirillum* application when compared to control plants. The significant increasing in protein content is due to the increase the percentage of 'N" and 'P' in plants [32]. It was found that organic acid of soils increased the plant uptake of P form a water soluble and also the release of organic acids both sequester cations and acidity. The microenvironment near the roots is through to be major mechanisms of 'P' uptake as well as Mn, Fe and Zn by plants and AM fungi. Increased levels of protein in the inoculated plants could be attributed to either the presence of fungal proteins stimulation of protein synthesis in the host plant [24]. It also observed by Arines *et al.* [33] in red clover. Nanthakumar and Veeraraghavathatham [34] in brinjal and Govindarajan and Thangaraju [35] in chilli.

The nutrient uptake was more in duel inoculation of *G. mosseae* + *Azsopirillum* application of black gram plants. AMF symbioses have been shown to benefit growth of many field crops in large part due to the extensive hyphal network development in soil, more efficient exploitation of nutrients, and enhanced plant uptake. They improve nutrient uptake, especially P, and also uptake of micronutrients such as zinc or copper; they stimulate the production of growth substances and may reduce stresses, diseases or pest attack [22].

The role of AMF on nutrient uptake (N, P and microelements), on the growth of AM crops, as well as on possible mechanisms of nutrient uptake, have been widely studied, as Al-Karaki [36], Cardoso and Kuyper [37], and Cavagnaro [38]. It is now generally recognized that AMF enhance the uptake of nitrogen (N) and of relatively immobile soil nutrients such as phosphorus (P), sulfur (S), copper (Cu), zinc (Zn), and boron (B) Martin *et al*. [39].

AMF formation is known to enhance nodulation and N_2 fixation by legumes [41]. Mycorrhizal and other symbioses often act synergistically on infection rate, mineral nutrition and plant growth [40]. The positive fungal effect on plant P uptake is beneficial for the functioning of the nitrogenase enzyme of the microbial symbiont leading to a higher N_2 fixation and, consequently to a better root growth and mycorrhizal development [42].

It concluded of my research article for better understanding of soil system would probably lead to a better management of AMF+ *Azospirillum,* contribution to soil fertility and, more sustainable agriculture, even in high yielding crop productions.

Table.1 Effects of *Glomus mosseae* and *Azospirillum* on the growth and biochemical content of Black gram *Vigna mungo* (L.) Hepper on 50th days of plants.

Treatments	SL (cm)	RL (cm)	No. of L	LA (cm^2)	No. of. RN	FW (mg/g fr. wt.)	DW (mg/g dr. wt.)	T.Chl (mg/g fr. wt.)	T.Sugar (mg/g fr. wt.)	Protein (mg/g fr. wt.)
T1	34.2 ± 1.71	22.5 ± 1.125	38.2 ± 1.91	29.7 ± 1.485	51.2 ± 2.56	40.48 ± 2.024	18.43 ± 0.97	3.22 ± 0.161	3.39 ± 0.17	9.5 ± 0.47
T2	37.7 ± 1.85	24.8 ± 1.24	41.5 ± 2.075	33.5 ± 1.675	68.5 ± 3.425	42.1 ± 2.105	19.82 ± 0.091	3.89 ± 0.1945	5.98 ± 0.30	11.21 ± 0.57
T3	38.8 ± 1.89	26.3 ± 1.315	44.2 ± 2.21	35 ± 1.75	77.6 ± 3.88	43.85 ± 2.1925	21.5 ± 1.175	4.08 ± 0.204	6.31 ± 0.32	14.79 ± 0.74
T4	42.7 ± 1.91	29.8 ± 1.49	47.4 ± 2.37	37.2 ± 1.86	81.5 ± 4.075	48.52 ± 2.426	23.64 ± 1.282	4.6 ± 0.23	7.6 ± 0.38	18.28 ± 0.92

± Standard deviation

T1 – Control, T2 = *Azsopirillum,* T3 = AMF, T4 = AMF + *Azospirillum.*

S.L=Shoot length R.L = Root Length No. of L= Number of Leaves LA= Leaf Area FW= Fresh wight No. of. RN= Number of root nodules ; DW= Dry weight; T. Chl= Total Chlorophyll; T.sugar= Total Sugar.

Table.2 Effects of *Glomus mosseae* and *Azospirillum* on the Nutrient content (mg/g dr. wt.) of Black gram *Vigna mungo* (L.) Hepper on 50th days of plants.

Treatments	N	P	K	Ca	Mg	S	Zn	Cu	Mn	Fe
T1	89.6 ± 4.45	5.5 ± 0.275	0.58 ± 0.03	1.85 ± 0.092	0.89 ± 0.044	1.3 ± 0.11	1.66 ± 0.08	0.14 ± 0.007	0.45 ± 0.023	1.15 ± 0.057
T2	92.7 ± 4.63	6.2 ± 0.31	1.38 ± 0.07	1.54 ± 0.077	1.08 ± 0.054	1.89 ± 0.19	2.18 ± 0.11	0.15 ± 0.0075	1.01 ± 0.051	2.67 ± 0.1335
T3	96.8 ± 4.84	7.9 ± 0.395	2.48 ± 0.2	1.63 ± 0.081	1.74 ± 0.09	2.05 ± 0.22	2.95 ± 0.15	0.24 ± 0.012	1.91 ± 0.096	2.98 ± 0.149
T4	99.2 ± 4.96	8.5 ± 0.425	3.63 ± 0.181	2.48 ± 0.124	2.03 ± 0.102	2.52 ± 0.26	3.68 ± 0.18	0.32 ± 0.016	2.68 ± 0.134	3.79 ± 0.190

± Standard deviation

T1 – Control, T2 = *Azsopirillum;* T3 = AMF T4 = AMF + *Azospirillum.*

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