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Infectivity and Efficacy of *Glomus fasciculatum* and *Acaulospora leavis* on the Growth and Nutritional Factors of *Vigna radiata* (L.) R. Wilczek

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Abstract: Arbuscular Mycorrhizal fungi (AMF) are known to form symbiotic association with plants and provide nutrients to plants for return of carbon source. In the present study a green house experiment was conducted to investigate the effect of dual inoculation with two AMF, viz. *Glomus fasciculatum* and *Acaulospora leavis,* on growth and nutritional factors of *Vigna radiata* (Green gram) in sterile (st) and unsterile (ust) soil condition. The results revealed that, there was an increase in plant growth parameters like, fresh and dry weights of shoot, total nitrogen, phosphorus contents, total protein and seed soluble protein in Ust + *A. leavis* inoculated plants compared to the control and between the treatments. In unsterilized soil, both *Glomus fasciculatum* and *Acaulospora leavis* showed 99% colonization. Whereas, in sterilized soil *Glomus fasciculatum* showed 92% colonization, while *Acaulospora leavis* showed 95% colonization. This indicates that AMF have different colonization strategies apart from nitrogen and phosphorus contents in the soil. The results of seed storage proteins revealed that, in *G. fasciculatum* inoculated sterile soil, there is slightly increase in protein concentration (0.58 mg/100mg) compared to both controls (0.425 mg and 0.566 mg/100 mg). The other treatments did not show any significant increase in total soluble seed protein. The present study suggested that *A. leavis* may be used as bio-inoculant in sustainable agriculture to increase the yield of Green gram.

Key words: AMF · Glomus fasciculatum · Acaulospora leavis · Vigna radiata · Seed storage protein

INTRODUCTION

Arbuscular mycorrhiza is a fungus of Glomeromycota [1] which from symbiotic association with plants. The primary benefit for symbiotic plants is an improved nutrition as AMF provides plants with nutrients, in particular phosphorus [2]. In agricultural systems the symbiosis is of great importance to plant health and crop yield [3]. It increases the photosynthetic rate and in leguminous plants the nitrogen fixation ability is also increased [4]. Some species of AM fungi have the capacity to break down phenolic compounds in soil which can interfere with nutrient uptake [5]. Root colonization by AM fungi provides protection from parasitic fungi and nematodes [6]. Non-nutritional benefits to plants due to changes in water relationships, phytoharmone levels, carbon assimilation, etc. have been observed in mycorrhizal plants [7, 2]. The most important benefit of AM association with leguminous plant is the absorption of phosphates and other nutrients from the soil [8]. The stimulation of nodulation and nitrogenase activity is often attributed to increased phosphorus status of mycorrhizal plants [9].

AM fungal association and their effects on different crops is currently of great interest due to important role played by different crops, especially by legumes and cereals in enriching the soil fertility. But agricultural practices have a negative impact on the AM fungal association and agricultural soil contains significantly low levels of AM fungal association, particularly in terms of numbers of species [10].

From the above mentioned beneficial aspects, it is evident that AMF are one of the indispensible parts of agricultural ecosystems. Hence, two dominant AMF species which were isolated and multiplied by trap culture method and were employed to express their efficiency on green gram (*Vigan radiata*), particularly to study the growth and nutritional factors, as green gram is one of the economically important pulse crop of India.

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Table 1: Physico-chemical parameters of soil used in pot experiment

Sl. No.	Sample Name	pH	EC mmhos/cm	OC (%)	Available P (kg/ha)	Available K (kg/ha)
1.	Sterilized	7.67	0.44	0.51	22.89	538
2.	Unsterilized	7.42	0.56	0.97	19.41	493

MATERIALS AND METHODS

Soil Chemical Analysis: The physico-chemical properties of experimental soil (sterilized and unsterilized) were analyzed at Central Sericulture Research and Training Institute (CSRTI), Mysore, Karnataka, India (Table 1).

Experimental Design: The experiment was setup during the month of February to April 2011 in poly house at Department of Studies in Botany, Manasagangotri, University of Mysore, Mysore, Karnataka. Green gram seeds (cultivar ART) were procured from Agricultural Produce Marketing Corporation, Mysore. Two isolates of AM fungi (*Glomus fasciculatum* and *Acaulospora leavis*) were obtained from Mycorrhiza Lab, Department of Studies in Botany, University of Mysore, Mysore, Karnataka. Potting medium consists of two different medium (mixture of sterile soil + sand and / or unsterile soil + sand) in 3:1 ratio was prepared. Surface sterilized green gram seeds were sown at the rate of 5 seeds per pot. The experimental set-up carried out were; sterile soil + sand (positive control), sterile soil + sand + AMF (*Glomus fasciculatum*), sterile soil + sand + AMF (*Acaulospora leavis*), unsterilized soil + sand (negative control), unsterilized soil + Sand + AMF (*Glomus fasciculatum*), unsterilized soil + sand + AMF (*Acaulospora leavis)*. About 200gm of AM fungal inoculum was added to respective pots (contains soil with spores and root bits). All the pots were placed in poly house condition and fertilized with Hoagland's solution (once in ten days) and plants were watered regularly to maintain moisture.

Harvests and Analyses: Plants were harvested when they attained the age of 90 days i.e. when the beans were in their late grain filling stage.The pots were subsequently inverted and roots separated from the soil, taking care without damaging the root. The root portion washed in running tap water to remove all the adhering soil particles and weighed before selecting root bits for staining. The cleaned roots were cut into 1cm long pieces and stained with trypan blue, according to the procedure described by Philips and Hayman [11]. The assessment of mycorrhizal infection was done by the simple slide method [12]. A minimum of 100 root segments were used for the enumeration and percent colonization of AM fungi and calculated using the following formula;

$$\% \text{ Colonization} = \frac{\text{Total number of root samples colonized}}{\text{Total number of root segments observed}} \times 100$$

The root and shoot portions of the plants were separated and gently pressed in folds of filter paper to remove water. The fresh weights of all the treatments were recorded. Later the samples were wrapped in paper and placed in hot air oven at 72°c for 48 hrs, removed and cooled in desiccators and reweighed to get the dry weight.

Estimation of Nitrogen: Total nitrogen was determined by Kjeldhal method [13]. The acid digested sample was transferred into the distillation flask. 2ml sodium hydroxide-sodium thiosulphate mixture prepared by dissolving 50g of sodium hydroxide and 5g of hydrated sodium thiosulphate in 100 ml of water and steam distilled. The distillate was titrated against 0.02N hydrochloric acid. The end point was chosen as the appearance of pink colour.

Estimation of Phosphorous: Estimation of phosphorus was carried out by Colorimetric Method [14]. 2ml of digested sample extract taken in 25ml volumetric flask. Few drops of 2,4-dinitrophenol indicator were added and neutralised the contents with 4N ammonia by adding few drops. Excess of ammonia was neutralised with 2N H_2SO_4 and the volume was adjusted to about two third of the flask with water. 1 ml of sulphomolybdate solution was added into it and the neck of the volumetric flask was washed with distilled water and 0.5 ml of freshly prepared stannous chloride solution was added. The contents were mixed thoroughly and the volume was made up to 25ml. Within 4 to 20 min the absorbance at 660 nm was recorded using a spectrophotometer. Same procedure was followed to prepare a standard curve containing 0.2 ppm to 1.0 ppm phosphorus. The amount of phosphorus in the sample was measured comparing to the standard curve and results were expressed as ppm phosphorous/gram dry wt. of the sample.

Estimation of Chlorophyll: The chlorophyll content in fresh leaves before harvesting was done by acetone extraction method [15]. 1 gram fresh leaves were cut into small pieces and homogenise in a mortar with excess acetone, with a pestle. The supernatant was decanted and filtered on Buchner funnel through Whatman No.42 filter paper. 80% acetone was added and repeated the extraction. The filtrates were pooled in volumetric flask. The absorbance of the extract was recorded at 645nm and 663nm.

Estimation of Total Proteins: The dry seeds without coat were ground with pestle and mortar and the fine powder produced (100 mg) was added to 1ml of 50 mM Phosphate buffer (pH 7.8) centrifuged at 10,000 g for 20 minutes to clarify the supernatant and finally stored at –20°C. The supernatant was used for quantification and electrophoretic profiles [16]. A colorimetric protein assay was done. 5ml of Protein solution was added to 5ml of Bradford reagent and the absorbance was measured at 595nm. A Bovine Serum Albumin (BSA) dilution curve was used as standard [17].

RESULTS AND DISCUSSION

The percentage of *Glomus fasciculatum* and *Acaulospora leavis* colonization revealed that both the bio-inoculants were able colonize slightly higher in plants grown in unsterilized soil when compared to plants grown in sterilized soil. In unsterilized soil both *Glomus fasciculatum* and *Acaulospora leavis* showed 99% root colonization. Whereas, in sterilized soil *Glomus fasciculatum* showed 92% root colonization, while *Acaulospora leavis* showed 95% colonization (Table 2). The efficacy of AM fungi inoculation was assessed by recording fresh weight and dry weight at 90 days old green gram plants. The results clearly elucidate that, among the two bio-inoculant used *A. leavis* was found effective in enhancing the shoot and root biomass compared to the control treatment. *G. fasciculatum,* was able to slightly enhance the total biomass; especially in unsterilized soils when compared to control (Table 3).

The root fresh and dry weight results revealed that the total biomass of crop plants grown in unsterilized soil significantly increased in both bio-inoculated crop plants when compared to non-inoculated and control plants (Table 3). It is evident that the plants inoculated with bio-inoculants increased growth and biomass of the green gram crop plant over control plants. The chlorophyll estimation results showed that the AM fungi inoculated plants did not increase chlorophyll content compared to the control plants. The concentration of chlorophyll was 25.8236 mg/g in plants grown in sterile soil and in unsterile soil plants it was recorded as 25.605 mg/g. Whereas, in all inoculated plants, the chlorophyll concentration was significantly less than controls plants, with only *G. fasciculatum* inoculated sterile soil showed 22.281 mg/g concentration of chlorophyll in the leaves (Table 4 and Fig. 1). *A. leavis* treated plants (Sterile soil), which showed high P concentration (80 ppm/g dry shoot) compared to control plants (Table 4 and Fig. 1). In sterilized soil control

Table 2: Percent root colonization of AM fungi

Experimental plant type	% colonization of roots.
St + *Glomus fasciculatum*	92
Ust + *Glomus fasciculatum*	99
St + *Acaulospora leavis*	95
Ust + *Acaulospora leavis*	99

Table 3: Fresh weight and Dry weight of the 90 days old green gram plants:

	Shoot weight		Root weight	
Experimental plant type	Fresh weight (gm)	Dry weight(gm)	Fresh weight (gm)	Dry weight (gm)
St + control	38	20	4	0.720
Ust + control	40	20	4	0.610
St + *G. fasciculatum*	57	22	6	2.246
Ust + *G. fasciculatum*	17	12.5	4	0.735
St + *A. leavis*	52	24	15	3.501
Ust +*A. leavis*	81	34.5	7	2.201

Table 4: Concentration of Chlorophyll, Phosphorous, Nitrogen and Total seed soluble protein in different treatments

Experimental plant type	Chlorophyll (mg/g of dry shoot powder)	Shoot phosphorus (ppm/g of dry shoot powder)	Shoot Nitrogen (mg/g of dry shoot powder)	Total seed soluble protein (mg/100 mg of dry seed powder
St + control	25.8236	25	1.3113	0.425
Ust + control	25.605	5	1.5674	0.566
St + *G. fasciculatum*	22.281	5	1.8023	0.58
Ust + *G. fasciculatum*	15.51	20	1.1288	0.53
St + *A.Leavis*	12.254	80	1.6325	0.52
Ust +*A. Leavis*	14.3	10	1.2718	0.48

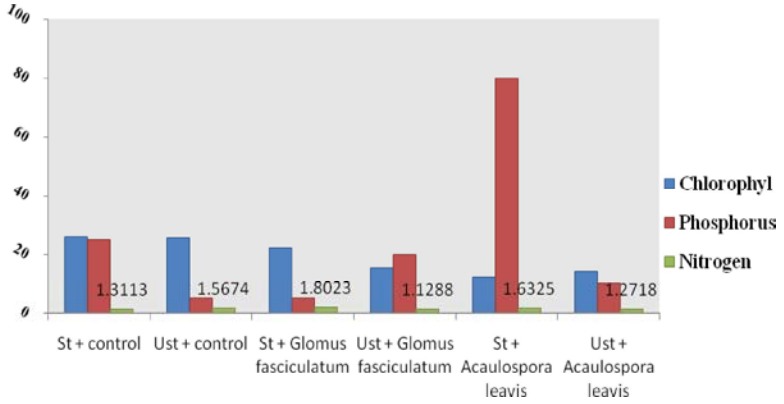

Fig. 1: Concentration of Chlorophyll, Phosphorous, Nitrogen and Total seed soluble protein in different treatments

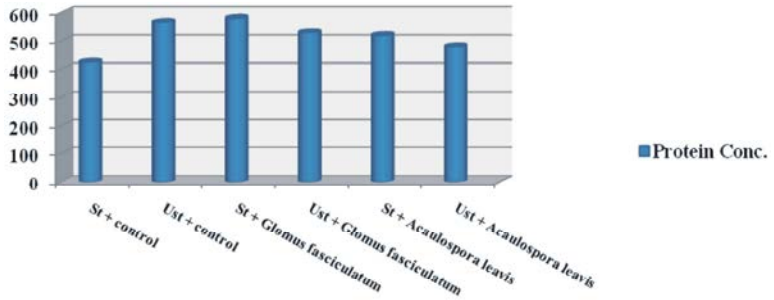

Fig. 2: Concentration of total seed soluble protein

plants P concentration was 25 ppm/g dry shoot and in unsterilized soil control plants, 5 ppm/gm dry shoot P concentration was recorded. Sterilized soil inoculated with *G. fasciculatum* showed 5 ppm/g dry shoot while in unsterilized plants the P concentration recorded as 20 ppm/g dry shoot. In unsterilized soil inoculated with *A. leavis* P concentration was recorded as 10 ppm/g dry shoot (Table 4 and Fig. 1).

In the present study, the concentration of nitrogen observed in all the treatments did not show any considerable variation compared to control. However, the sterilized soil inoculated with both bio-inoculants showed slightly higher (1.8023 mg/g dry shoot) concentration when compared to control. Nitrogen concentration was slightly reduced in plants inoculated with both bio-inoculants in unsterilized soil condition (Table 4 and Fig. 2). Estimation of seed storage proteins revealed that, *G. fasciculatum* inoculated plants harboured a slightly increase in protein concentration (0.58 mg/ 100 mg) compared to both controls (0.425 mg and 0.566 mg/ 100 mg). The other treatments did not show any significant increase in total soluble seed protein compared to control (Table 4 and Fig. 2).

Most of the cultivated plant species are able to form AM symbiosis. Various workers demonstrated that AM fungi can play an important role for increasing the plant health and helps in acquiring nutrition. In the present study, two AM fungi, *Acaulsopora leavis* and *Glomus fasciculatum* were used to evaluate the efficacy for increasing the growth and nutritional status of the green gram (*Vigna radiata*) in two edaphic systems, viz. sterilized soil and unsterilized soil.

Experiment conducted in sterilized soil would helps us to evaluate the indigenous effects of AMF on host plant and their growth parameters, while the experiment conducted in unsterilized soil helps us to evaluate the direct effects of AMF on host plant in the presence of other soil microbiota, which is similar to natural conditions. This would also explore the beneficiary aspects of AMF inoculums in natural or agricultural ecosystems which is an important step in determining the effects of AMF inoculums upon agricultural productivity and eventual development of management strategies using these fungi.

Percent colonization results reveals the higher percent of colonization by both the bio-inoculants with their host plants in both the experimental soils, i.e. sterilized and unsterilized soils. In sterilized soil conditions both *G. fasciculatum* and *A. leavis* were able to colonize about 92 and 96%, respectively, while the percent colonization of the AMF in unsterilized soils were 99% which indicates that host plants roots were

completely colonized by mycorrhiza. It is also assumed that unsterilized soil may contain different types of AMF species and 99% colonization would be attributed to this aspect as the other species of AMF might have colonized the roots of experimental host plants (Table 2). The soil physico-chemical parameters were not seems to have any influence on the percent colonization, because almost all the parameters were considerably equal in both soil conditions (Table 1).

The present study also revealed that, both the AMF could able to significantly increase the biomass of host plants compared to control plants. Amongst all the treatments, *A. leavis* performed well in unsterilized soil plants which showed highest shoot and root weight, followed by *G. fasciculatum* inoculated plants into sterilized soil condition (Table 3). The total chlorophyll content does not seem to be influenced by AMF colonization. Control plants exhibited slightly large quantity of chlorophyll content compared to AMF inoculated plants (Table 3).

It is evident that AMF mainly increase phosphorus uptake and moderately nitrogen content. In the present study, it was observed that phosphorus uptake was significantly increased only in the *A. leavis* inoculated plants in sterilize soil condition and also to some extent in unsterile soil condition inoculated with *G. fasciculatum* palnts compared to control (Table 4). In contrast with the earlier report, where *A. leavis* did not influenced much on shoot growth and phosphorous content in DWR-162 var. of *Triticum aestivum* L. compared to *Glomus fasciculatum* [18], it has been recorded that *A. leavis* influenced significantly in assimilating phosphorus and increasing the plant biomass. This could be attributed to the host specificity and environmental effects of individual AM fungi. Further, it is also noted that the beneficial impact of AM fungi on plant mineral content and secondary metabolite contents depends not only on the AM fungal species but also on plant genotype and fertilization regime [19-21].

CONCLUSION

The present work demonstrates the efficacy of AMF in two different types of treatments, sterilized soil plants could be depicted as agricultural soils which contains very low levels of AMF abundance or do not possess AMF population and unsterilized soil as normal agricultural soil. Our results signify the effectiveness of AMF in unsterilized soil where increased plant growth was observed. It also signifies the potentiality of AMF bio-fertilizers in agri-ecosystems. Therefore selected AMF species could be used as bio-fertilizers in agricultural practices, in order to get higher yield and plant biomass.

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