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RESEARCH ARTICLE

Title: Effect of Arbuscular mycorrhizal fungi (AM fungi) and *Rhizobium* on the nutrient uptake of pigeon pea plant.

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Abstract

The aim of the present study was to investigate the effect of Arbuscular mycorrhizal fungi (*Glomus fasciculatum*) and *Rhizobium* on the nutrient uptake of pigeon pea, a legume. A pot culture experiment was conducted in the Department of Life Science and Bioinformatics, Assam University, Silchar. The results showed that there was an overall increase in the nutrient (N, P, K, Fe, Cu, Zn and Mn) uptake in all the treated plants as compared to control. But dual inoculation with *Glomus fasciculatum* and *Rhizobium* had a remarkable effect on the nutrient uptake of pigeon pea plant. Cu was not detected in untreated (control) plant. But all the treated plants exhibited the uptake of copper. The combined application of microsymbionts revealed a synergistic effect in terms of nutrient uptake in legumes.

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INTRODUCTION

Pigeon pea is a major protein rich pulse crop widely cultivated throughout India. Among all the pulse crops grown in India, pigeon pea occupies the most priority in pulse production. It has got immense nutritional and medicinal value. So it is necessary to increase the rate of its production to meet the demand of growing population. There are possibilities of increasing the rate of production by the application of biofertilizer. Both Arbuscular Mycorrhizal fungi (AM fungi) and *Rhizobium* act as biofertilizer having the ability to convert nutritionally important elements from unavailable to available form through biological process (Vessey, 2003). AM fungi play a major role in increasing the productivity of leguminous crops (Matias, Pagano, Muzzi, Oliveira, Carneiro, Horta, Nadja, and Scott, 2009). Most of the legumes possess two types of microbial symbionts namely AM fungi and *Rhizobium*, a nitrogen fixing soil bacteria thereby establishing triple association capable of supplying nitrogen and phosphorus to the plants (Kawaguchi & Minamisawa, 2010). Micronutrients especially Zn, B and Mo help in protein synthesis and carbohydrate metabolism. Therefore, for better grain quality of seed grain, management of micronutrients needs special attention. Barak valley of North-East India, being a part of mega diversity hot spot known as Indo Burma hot spot region harbour a rich biological diversity. But still it is legging behind in the production of pigeon pea in comparison with the rest part of India. A combination of legumes, AM fungi and *Rhizobium* can bring a significant improvement in the nutrient uptake of plants thereby increasing overall growth and yield of plants. Therefore, the present study was undertaken to investigate the effect of dual interaction of AM fungi (*Glomus fasciculatum*) and *Rhizobium* in the nutrient uptake of pigeon pea plant.

Materials and methods

The study was carried out in the Department of Life Science and Bioinformatics of Assam University located in Silchar (Barak valley), between 23^0N to 24^0N latitude and between 92^0E and 93^0E longitude. Four treatments were performed in the present study as followed

1) Inoculation with *Glomus fasciculatum* alone
2) Inoculation with *Rhizobium* alone
3) Dual inoculation with *Glomus fasciculatum* and *Rhizobium*
4) Uninoculated plants considered as control

Preparation of inoculum

Inoculation with *Glomus fasciculatum*

Glomus fasciculatum was isolated from the rhizospheric soil of pigeon pea by wet sieving and decanting technique (Gerdemann & Nicolson, 1963) and multiplied on the roots of *Zea mays* plants. The plants were allowed to grow for three months after which the shoots were severed and the substrate containing root fragments, mycelium and spores were collected and air dried and used as inoculum. Healthy seeds of pigeon pea were surface sterilized with 0.2% mercuric chloride and were washed several times with sterile distilled water. The seeds were germinated in the sterilized soil under dark condition. The seedlings were allowed to grow for 10 days. Seedlings of uniform height were transplanted in plastic pots (one seedling in each pot) containing 2kg oven sterilized mixture of sand and soil in the ratio 1:1. Seedlings were inoculated with mycorrhizal inoculum (100g/pot) containing maize root fragments, mycelium and spores.

Inoculation with *Rhizobium*

The *Rhizobium* isolates were obtained from root nodules of pigeon pea. Healthy, pinkish, well formed, unbroken nodules were collected from the roots of pigeon pea. The nodules were surface sterilized for 3 minutes in 0.1% mercuric chloride and washed repeatedly with sterile water. Individual nodules were crushed with sterile glass rods in a small aliquot of sterile water and milky fluid was streaked on to sterile Yeast Extract Manitol Agar (YEMA) containing Congo red (2.5 ml of 1% Congo red in 100ml YEMA). The plates were incubated at 28^0C for 5 days. Colonies were selected and streaked on YEMA for purity and pure culture of isolated bacteria was subjected to morphological and biochemical (growth on Hofers alkaline medium, growth on Glucose peptone agar) and nodulation test for characterization as described by Subba Rao (1999). For inoculation, healthy, sterilized seeds of pigeon pea (about 50) were suspended in 20- 40 ml thick suspension of *Rhizobium* for 30 minutes. The seeds were air dried for 30 minutes in sterile petri plates. The seeds were germinated in sterilized soil under dark condition. The seedlings were allowed to grow for 10 days. Seedlings of uniform height were transplanted in plastic pots (one in each pot) containing 2Kg oven sterilized mixture of sand and soil in the ratio (1:1).

Dual inoculation with *Glomus fasciculatum* and *Rhizobium*

For combined inoculation with *Glomus fasciculatum* and *Rhizobium*, 10 days old seedlings grown in dark condition (seeds were pre inoculated with *Rhizobium*) were transplanted in plastic pots (one in each pot) containing mycorrhizal inoculum.

Control(Untreated plants)

For control, sterilized seeds were grown in dark condition for 10 days and seedlings of uniform height were transplanted into pots containing 2 Kg of mixture of sterilized soil and sand mixed in the ratio (1:1).

The experiment was laid out in randomized complete block (RCB) design. The plants were watered everyday.

Estimation of nutrient contents

Nutrient contents of the plants were measured after 120 days of inoculation of the plants. Three replicates at random from each treatment were selected for estimation of nutrients uptake. Shoots and roots of the plants were dried in the oven at 80^0C for 48 hours. Dried plant materials were converted to ashes for estimation of nutrient contents. Nitrogen contents of plant ash were determined by Alkaline permanganate method (Subbiah & Asija, 1956) and phosphorus contents were determined by Spectrophotometric method (Bray & Kurtz, 1945).

For estimation of Fe, Cu, Mn, Zn and K of different treatments and control sets, dried plant materials were digested in diacid mixture (nitric acid: perchloric acid, 4:1) and nutrient contents of dried plant materials were determined by Atomic Absorption Spectrophotometer (SAIF, Shillong).

Results and discussions

On Yeast Extract Manitol Agar (YEMA) media, the colonies of bacteria appeared as circular, cream coloured, raised translucent structure. On Yeast Extract Mannitol Agar media containing congo red, colourless

colonies appeared indicating that isolates were *Rhizobium* (Dye, 1979; Vincent, 1970).The organisms could not grow at pH 11.0 in Hofer' alkaline medium which also indicated that the isolates were *Rhizobium* (Gaur & Sen, 1981). Besides, slow growth on Glucose peptone agar media and formation of nodules on the roots of bacterized plants confirmed that isolates were *Rhizobium.*

The effects of lone inoculation of plants with *Glomus fasciculatum* and *Rhizobium* and their combined inoculation effect are presented in the **Table 1**. The data presented in the Table **1** revealed that lone inoculation of the plants with both *Glomus fasciculatum* and *Rhizobium* and their combined inoculation resulted in considerable increase in the nutrient uptake as compared to untreated plant (control). There was an overall increase in nutrient contents in the treated plants as compared to control.

Table 1. Nutrient contents of pigeon pea as influenced by dual inoculation with *Rhizobium* and *Glomus fasciculatum*

Microbial inoculants	N	P	K	Cu	Fe	Mn	Zn
Control	3.04	0.98	1.30	ND	8.61	1.21	0.34
Rhizobium	3.18	1.87	1.33	0.03	8.65	2.02	1.40
VAM fungi	3.26	2.03	6.96	0.06	10.77	4.3	6.00
VAM+ Rhizobium	3.34	2.10	7.12	0.98	11.50	4.35	6.11
CD (p=0.05)	1.02	0.02	0.06	0.99	0.01	0.01	0.12

ND= not detected.

The results clearly indicated that N, P and K contents of plants lonely inoculated either with *Glomus fasciculatum* or *Rhizobium* are higher than those of control (untreated). Maximum N, P and K contents were recorded in the plants dually inoculated with *Rhizobium* and *Glomus fasciculatum.* Inoculation of legumes with *Rhizobium* increase nodulation of legumes causing more nitrogen fixation and making it available to the plants. Moreover, it also increases rhizospheric microflora viz. acid producers and phosphate solubilizers causing more available phosphorus (Lipman & Conybeare, 1936). Abd-Alla and Omar (2001) reported that *Rhizobium* inoculation significantly improved nitrogen and phosphorus contents in faba bean plant which was also substantiated by our present investigation. Arbuscular mycorrhizal fungi supported nitrogen fixation by providing legumes with phosphorus and other immobile nutrients (Clark & Zeto, 2000). Yaseen, Burni, and Hussain, (2011) also reported that AM fungi contributed to increase in the uptake of phosphorus and other micronutrients. Talaat and Abdallah (2008) reported that Arbuscular mycorrhizal fungi were more effective when legumes were inoculated with *Rhizobium* resulting in an increase in N, P and K contents. So far increase in K content is concerned, it might be due to overall vigour and increased cation exchange capacity of the inoculated plant roots (Bhat, Rashid, Rasool, Mahdi, Haq, and Bhat, 2010). Many other reports are also available on the increased N, P and K contents in legumes due to dual inoculation with AM fungi and *Rhizobia* (Chakrabarty, Chatterjee, and Dutta, 2007; Soliman, Shanan, Massoud, and Swelim, 2012). which provide support to our findings.

Cu was not detected in the untreated plants in our present study. The results revealed that lone inoculation with either *Glomus fasciculatum* or *Rhizobium* had insignificant but positive effects on the uptake of Cu. There was an overall increase in Cu content in all the treated plants. But dual interaction of *Glomus fasciculatum* and *Rhizobium* significantly enhanced the uptake of Cu. Tavasolee, Aliasgharzad, Salehijouzani, Mardi, and Asgharzadeh, (2011) reported that dual inoculation of legumes with Arbuscular mycorrhizal fungi and *Rhizobium* enhanced N, P, Zn, Fe and Cu contents which corroborated our findings.

Fe, Mn, Zn also increased considerably in all the treated plants as compared to control in our present study. Plants dually inoculated with *Glomus fasciculatum* and *Rhizobium* exhibited maximum uptake in Fe, Mn and Zn. AM fungi have the capacity to increase the uptake of many nutrients such as nitrogen, phosphorus sulphur, zinc, iron and copper (Mathur &Vyas, 2000). As AM fungi are mutualistic symbionts, they can drain up to 20% of photosynthetic carbon (Jacobsen & Rosendahl, 1991) and in return provide the plants with large amount of nutrients (phosphorus, nitrogen, potassium, zinc etc) and water from the soil. Tavasolee *et al.*, 2011 suggested that effective AM fungi can enhance the performance of *Rhizobium* and vice versa which were supported by the results of our present study.

Conclusion

The results obtained with *Glomus fasciculatum and Rhizobium* in conjugation might be attributed to overall improvement of growth, development of inoculated pigeon pea plants in terms of better nutrient availability, nutrient uptake etc. Thus, overall findings have shown the synergistic effects of co inoculation of AM fungi and *Rhizobium* in the nutrient uptake of pigeon pea plants indicating that the combination may have a promising role to enhance the productivity of pigeon plant.

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