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Effects of Dual Inoculation of Arbuscular Mycorrhizal Fungi (*Glomus fasciculatum*) and *Rhizobium* on the Growth and Nodulation of Pigeon pea (*Cajanus cajan* L.)

Sujata Bhattacharjee* and G.D. Sharma

Department of Life Science, Assam University, Silchar - 788 011, India.

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A pot culture experiment was conducted to investigate the effects of dual inoculation of Arbuscular mycorrhizal (AM) fungi (*Glomus fasciculatum*) and *Rhizobium* species on the growth and nodulation of Pigeon pea (*Cajanus cajan* L.). Four treatments were conducted i.e., 1. Inoculation with *Glomus fasciculatum* alone. 2. Inoculation with *Rhizobium* alone. 3. Inoculation with *Glomus fasciculatum* and *Rhizobium* in combination. 4. Control i.e. without inoculation. The results revealed that *Glomus fasciculatum* markedly increased the plant growth in terms of plant height, number of leaves, plant fresh weight and dry weight. But maximum growth was observed in the plants dually inoculated with *Glomus fasciculatum* and *Rhizobium.* Higher counts of nodules were observed in the plants inoculated dually with *Glomus fasciculatum* and *Rhizobium* in comparison with the plants inoculated with *Rhizobium* alone.

Key words: Arbuscular mycorrhizal fungi, *Rhizobium*, Symbiotic association, Dual inoculation, Nodulation.

Mycorrhizal association between fungi and roots of higher plants and nitrogen fixing interaction between the *Rhizobia* and legumes are the most commonly studied symbioses (Newman and Reddell, 1987)[1]. Both the arbuscular mycorrhizal(AM) fungi and *Rhizobium* act as biofertilizers and have the unique ability to convert nutritionally important elements from unavailable to available form through biological processes (Hedge, 1999, Vessey, 2003)[2-3]. Utilization of biofertilizers in the cultivation of medicinal and leguminous plants has gained significant importance in recent years to reduce chemical input and to raise the soil fertility and to improve the crop production. *Rhizobia* have the capacity to fix atmospheric nitrogen and thus add to the amount of nitrogen to the soil. The *Rhizobium* inoculants may be used as an alternative for urea (Karim, *et al*., 2001)[4]. Arbuscular mycorrhizal (AM) fungi can benefit the host plant by increasing the ability of root system to absorb and translocate phosphorus through an extensive network of external hyphae (Hayman, 1983)[5]. The development of symbiosis between AM fungi and most terrestrial plants are beneficial to both the partners (Miransari, 2010)[6]. Researches in the past few decades have established the fact that dual inoculation of AM fungi and *Rhizobium* have increased the plant growth, nodulation and yield in legumes (El-Ghandour *et al*., 1996, Subba Rao, 1999, Kumar

* To whom all correspondence should be addressed.
E-mail: bhattacharjree.sujata819@gmail.com

et al., 2001, Ammani, 2002, Chakrabarty *et al*.,2007, Talaat and Abdallah, 2008)[7-12].

Pulses contribute to the major and high protein component in Indian diet. Pulses contain a high percent of quality protein. Pigeon pea is one of the major protein rich pulse crops grown in most part of the country. Plants have got immense medicinal value and are also used as fodder for cattle. In Barak valley of Southern Assam, India, the rate of production of Pigeon pea is low in comparison with the other parts of the country. A combination of legumes, *Rhizobia* and mycorrhizal fungus can bring a significant improvement in plant growth through increased availability of phosphorus together higher nitrogen fixation in soil. Thus, the combination might prove the cheapest way to enrich the soil of Barak valley thereby increasing the rate of production of Pigeon pea in this region. Therefore, an attempt has been made in the present study to evaluate the effects of dual inoculation of AM fungi and *Rhizobium* on the growth and nodulation of Pigeon pea.

MATERIAL AND METHODS

The experiment was carried out in the Department of Life Science, Assam University, located in Barak valley of Southern Assam, India and between 23^0N and 24^0N latitude and between 92^0E and 93^0E longitude. Four different treatments were maintained in the present study which were as followed:

1. Inoculation with *Glomus fasciculatum* alone
2. Inoculation with *Rhizobium* alone
3. Inoculation with *Glomus fasciculatum* and *Rhizobium* in combination
4. Untreated plants considered as control

Inoculation with *Glomus fasciculatum* alone

Glomus fasciculatum was isolated from the rhizospheric soil of Pigeon pea by wet sieving and decanting technique (Gerdemann and Nicolson, 1963)[13] and multiplied on the roots of *Zea mays* plants. The plants were allowed to grow for three months after which the roots 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 mixture of sterilized soil under dark condition. The seedlings were allowed to grow for 10 days. Seedlings of uniform height were transplanted in polythene bags (one seedling in each bag) containing 2kg oven sterilized mixture of sand and soil in the ratio 1:1. Seedlings were inoculated with mycorrhizal inoculum containing maize root fragments, mycelium and spores.

Inoculation with *Rhizobium* alone

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 5 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) test for characterization as described by Subba Rao (1999)[8]. For inoculation, healthy, sterilized seeds of Pigeon pea (about 50) were suspended in 20- 40 ml thick suspension (10^9cells/ml) of *Rhizobium* for 30 minutes. The seeds were air dried for 30 minutes in sterile Petri plates. 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).

Inoculation with *Glomus fasciculatum* and *Rhizobium* in combination (dual inoculation)

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.

Untreated plants (control)

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.

Measurement of growth of seedlings

Growth parameters of seedlings in terms of plant height and number of leaves were measured after 30, 60, 90 and 120 days of transplantation. Fresh weight and dry weight of the plants were measured after 120 days of transplantation. For this, the roots were properly washed to remove adhering soil particles. The fresh weight of the roots and shoots were measued separately with the help of electronic balance. The shoots and roots were dried separately in the oven at 80^0C for 48 hours. The dried weight of the roots and shoots were taken with the help of Electronic balance. Numbers of nodules were counted after 30, 60, 90 and 120 days of transplantation.

RESULTS AND DISCUSSION

On YEMA (Yeast Extract Manitol Agar), the colonies of bacteria were circular, cream coloured, sticky and raised translucent structure. On YEMA containing congo red, colourless to faint colonies appeared indicating that the isolates were *Rhizobium* species (Dye, 1979, Vincent, 1970)[14-15]. The organism could not grow at pH 11.0 in Hofer's alkaline medium which also indicated that the isolates were *Rhizobium* (Gaur and Sen,1981)[16]. More over, the slow growth on the Glucose peptone agar media and formation of nodules at the root of the plants inoculated with bacteria confirmed that the isolates were *Rhizobia*.

The assessment of growth parameters recorded in the Table1 revealed that the plants showed varied response to various treatments. The overall growth of the treated plants was better as compared to untreated plants. Maximum average plant height, number of leaves, plant fresh and dry weight and nodules were recorded in the plants dually inoculated with *Rhizobium* and AM fungi which was followed by the lone inoculation with AM fungi. The untreated plants showed minimum growth in terms of plant height, leaf number, plant fresh and dry weight. Lone inoculation with *Rhizobium* enhanced the plant height by 18.6%, lone inoculation with *G.fasciculatum* enhanced the plant height by 23% and dual inoculation with *G.fasciculatum* and *Rhizobium* enhanced the plant height by 30.5 % over untreated plants.

Table 1. Effect of dual inoculation of *Glomus fasciculatum* and *Rhizobium* on the plant height, number of leaves, plant fresh weight and dry weight and nodules of Pigeon pea

Treatment	30 Days			60 Days			90 Days			120 Days				
(cm)	PI. Ht	No. of Leaves /PI	No. of Nodules /PI	PI. Ht (cm)	No. of Leaves /PI	No. of Nodules /PI	PI. Ht (cm)	No. of Leaves /PI	No. of Nodules /PI	PI. Ht (cm)	No. of Leaves /PI	Fresh wt./PI (g)	Dry wt./PI (g)	No. of Nodules /PI
Golmus fasciculatum	22.5	6.8	0	48.62	13.8	0	73.7	18.8	0	86.3	29.6	17.3	8.2	0
Rhizobium sp.	22	6.6	7.8	45.64	12.8	13.8	70.5	17.2	21.2	82.04	25.8	14.7	7.52	15.4
Glomus fasciculatum +*Rhizobium sp.*	24.5	7.8	11.6	52.62	16.2	18.2	78.8	20.4	27.6	91.64	32.6	20.24	10.46	19.2
Untreated (Control)	21	5.8	0	43	10.2	0	61.62	13.8	0	70.2	16.42	11.24	4.7	0
CD (p = 0.05)	1.25	NS	0.1	1.31	0.2	0.15	1.6	0.33	0.31	1.82	0.5	0.64	0.38	0.2

Plants inoculated with *Rhizobium* alone and those inoculated dually with G.fasciculatum and *Rhizobium* showed the presence of nodules at their root systems. Nodules were not detected in the roots of untreated plants (control) and the plants inoculated with *G.fasciculatum* alone. The number of nodules increased gradually from 30 days to 90 days but less number was recorded after 120 days of transplantation. Plants inoculated dually with *G.fasciculatum* and *Rhizobium* exhibited higher number of nodules in comparison with the plants inoculated *Rhizobium* alone and highest number of nodules were recorded after 90 days of transplantation.

The results highlighted that mutualistic double symbiosis by *Rhizobium* and AM fungi provided better growth than either of the single symbiotic microbial symbiosis with leguminous crop plants. The *Rhizobia* may augment the plant growth by providing products of dinitrogen fixation, either by direct bacterium-plant transport of fixed nitrogen or by slow transfer due to a gradual death and mineralization of the bacteria. The filamentous network of AM fungi promote bidirectional nutrient movement so that water and soil nutrient move to the plants and plant photosynthates flow to the mycelial network of mycorhizal fungi(Seguin *et al.*,.2003)[17]. The mutualistic double symbiosis might be responsible for interchange of carbon, phosphorus and nitrogen among the host, the fungus and bacteria resulting in the better plant growth in terms of plant height, leaf number, plant fresh and dry weight than either of the lone inoculation. Significant increase in plant dry weight due to tripartite association of rhizobia, mycorrhizal fungi and legumes was reported by earlier workers (El-Ghandour *et al.*, 1996; Rabie, 1998)[7, 18] which are in agreement with the findings of our present study.

The number of nodules increased gradually from 30 days to 90 days after transplantation and maximum numbers of root nodules were recorded after 90 days of transplantation. But number of nodules decreased after 120 days of transplantation which might be due to senescence and decay of nodules after the completion of growth phase of the crops. Highest number of nodules was recorded in the plants inoculated dually with *G.fasciculatum* and *Rhizobium*. The increase in the nodulation might be attributed to the effect of AM fungi on phosphorus solubilization which resulted in the better root development and nodulation. Both the nodulation and nitrogen fixation depend on the balanced supply of mineral nutrition, especially phosphorus. Mycorrhizal legumes have the benefit of harnessing the resources of phosphorus which is other wise unavailable to the plants. Mycorrhizal fungi are known to have an important role in improving growth; nodulation and nitrogen fixation in legume crops symbiotic with *Rhizobium* spp. (Barea and Azcon-Aguilar, 1983; Barea *et al*., 1992)[19-20] which indicated the existence of inter endophytic compatibility between AM fungi and *Rhizobium*.

Many reports are available on the increased growth and nodulation due to dual inoculation of AM fungi and *Rhizobium* (Subba Rao, 1999;Kumar *et al.*, 2001; Ammani, 2002 Chakrabarty *et al*., 2007; Talaat and Abdallah, 2008)[8-12] which were supported by the results of our present study. Bhat, *et al*., (2010)[21] have also reported that dual inoculation of AM fungi and *Rhizobium* had a significant on the nodulation in green gram.

Summing up, the dual inoculation of AM fungi and *Rhizobium* provided better growth in terms of plant height, number of leaves, plant fresh and dry weight and nodulation than either of the lone inoculation in Pigeon pea indicating that the combination may have a promising role to benefit the legume in the soil of Barak valley.

REFERENCES

1. Newman, E.I. and Reddell, P. The distribution of mycorrhizas among families of vascular plants. *New Phytol*, **106**: 745- 751. Oldroyd GED, Engstrom EM, Long SR. 2001. Ethylene inhibits the Nod factor signal transduction pathway of *Medicago truncatula. Plant Cell*, 1987; **13**: 1835-1849.
2. Hegde, D.M., Dwived, B.S. and Sudhakara, S.N. Biofertilizers for cereal production in India review. *Indian J. Agric.Sci.*, 1999; **69**: 73-83.
3. Vessey, J. K. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil*, 2003; **255**: 571- 586.
4. Karim, M.R., Islam, F., Akkas Ali, M. and Haque, F. On-farm trail with *Rhizobium* inoculants on lentil. *Bangladesh J. Agric. Res.*, 2001; **26**: 93-94.

5. Hayman, D. S., The physiology of vesicular arbuscular endomycorrhizal symbiosis. *Canadian J. Bot.*,1983; **61**: 944- 963.
6. Miransari, M. Contribution of Arbuscular Mycorrhizal symbiosis to plant growth under different types of soil stress. *Plant Biology*, 2010; **12**: 563-569.
7. El-Ghandour, I.A.,El-Sharawy, M.A.O. and Abdel-Moniem, E.M. Impact of vesicular arbuscular mycorrhizal fungi and *Rhizobium* on the growth and P,N and Fe uptake by faba-bean. *Fertilizer Research*, 1996; **43**: 43-44.
8. Subba Rao, N.S. Rhizobacterium and legume root nodulation. In Soil Microbiology, 1999; P.169. Oxford & IBH publishing Co. Pvt. Ltd. New Delhi.
9. Kumar, R., Gupta, P.P. and Jalali, B.L. Impact of VA-mycorrhiza, Azotobacter and Rhizobium on growth and nutrition of cowpea. *J.Mycol. Pl. Pathol.*, 2001; **31**(1): 38-41.
10. Ammani, K. Triple inoculation of a VAM fungus and two nitrogen fixing bacteria on two cereals. *Asian J. of Microbiol Biotech. Env. Sc.* 2002; **4**(1): 99-105.
11. Chakrabarty, J., Chatterjee, N.C. and Dutta, S. Interactive effect of VAM and Rhizobium on nutrient uptake and growth of *Vigna mungo*.*J. Mycopathol. Res.*, 2007; **45**(2): 289-291.
12. Talaat, N.B. and Abdallah, A.M. Response of Faba Bean (*Vicia fava* L.) to dual inoculation with *Rhizobium* and VA Mycorrhiza under *Different* Levels of N and P Fertilization. *Journal of Applied SciencesResearch*, 2008; **4**(9): 1092-1102.
13. Gerdemann , J.W. and Nicolson, T.H. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Br.Mycol. Soc.*, 1963; **46**: 235-244.
14. Dye, M. Functions and maintenance of Rhizobium collection. In Recent advances in biological nitrogen fixation, (ed. Subba Rao NS). Oxford and IBM publishing Co. New Delhi, 1979; pp. 435-471.
15. Vincent, J.M. A manual for the practical study of the root nodule bacteria, IBP Hand Book No. 15, Blackwell Scientific publications, Oxford, 1970.
16. Gaur, Y.D. and Sen, A.N. Cultural and Biochemical characteristics of root nodule bacteria of chickpea [*Cicer arietinum* (L.)]. Zbl Bakt II, 1981; Abst 136, 307-316.
17. Seguin, S., Desaulniers, N., Dalpe, Y. and Levesque, C.A. Development of AMF strains specific primers for detection in field-grown colonized roots. 4th Int. Conf. *Mycorrhizae*, Montreal Quebec Canada, August 10-15, 2003; No. 444.
18. Rabie, G.H. Induction of fungal disease resistance in *Vicia faba* by dual inoculation with *Rhizobium leguminosarum* and vesicular-arbuscular mycorrhizal fungi. *Mycopathologia*, 1998; **141**: 159-166.
19. Barea, J. M. and Azcon-Aguilar, C. Mycorrhizas and their significance in nodulating nitrogen-fixing plants. Adv. Agron., 1983; **16**: 1.
20. Barea, J. M., Azcon, R. and Azcon-Aguilar, C. Vesicular-arbuscular mycorrhizal fungi in nitrogen fixing systems. In: Norris J. R., Read, D. J. and Varma, A. K. (eds). *Methods in Microbiology*. Academic Press, London, 1992; pp.391-416.
21. Bhat, M.I., Rashid, A., Faisul-ur-Rasool, Mahdi, S.S., Haq, S.A. and Bhat. R.A. Effect of *Rhizobium* and Vesicular arbuscular mycorrhizae Fungi on Green gram (Vigna radiata L. Wilczek) under Temperate Conditions. *Research Journal of Agricultural Sciences*, 2010; **1**(2): 113-118.