JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2010. Vol. **4**(1), p. 409-414

Influences of Three Different Arbuscular Mycorrhizal Fungi on the Growth and Phosphorus Contents of *Cajanus cajan* L. Seedlings

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(Received: 02 September 2009; accepted: 11 October 2009)

The present study was undertaken to assess the effects of three different Arbuscular mycorrhizal fungi (AMF) namely *Glomus fasciculatum, Glomus aggregatum* and *Acaulospora scrobiculata* on the growth and phosphorus contents of *Cajanus cajan* L. seedlings at different inoculum potential (IP) levels. An initial inoculum potential level of 13000 infective propagules (1IP) per polythene bag determined by most probable number (MPN) method was used. Different inoculum potential levels were 1/2 (IP), 1(IP), 2(IP) and 4(IP). It was observed that plants inoculated with AM fungi showed significant increase in plant height, total dry weight, root colonization and phosphorus contents as compared to un inoculated ones. The plants inoculated with *Glomus fasciculatum* showed maximum increase in the plant growth, root colonization and phosphorus contents as compared to the plants inoculated with either of the two AMF. Plants varied in their growth response with different inoculum potential levels of AM fungi. Growth parameters showed an increasing trend with the increasing inoculum potential levels and maximum response was observed at an inoculum potential level of 4(IP).

Key words: Arbuscular mycorrhizal fungi, inoculum potential, phosphorus content, root colonization.

The arbuscular mycorrhizal fungi (AMF) are rhizospheric in nature that develop a nonspecific symbiotic association with the majority of vascular plants (Gianinazzi and Gianinazzi, 1986)[1]. Occurrence of AMF is ubiquitous. They are found in dense rain forest, open wood lands, scrubs, savanna, grasslands, heaths, sand dunes and semi deserts (Gerdemann, 1968)[2]. Over past few decades, there has been growing appreciation of the plants and microbes interaction especially arbuscular mycorrhiza in terrestrial ecosystem (Zhao *et al*., 2002, Muthukumar *et al.*, 2003)[3-4]. These root symbionts have gained immense importance in agriculture, horticulture, afforestation and land reclamation (Prasad, 2000, Charles *et al*., 2006 andJavot *et al*., 2007)[5-7]. Researches have established that AM fungi improve the growth of crop plants through uptake of nitrogen and phosphorus (Abott *et al*., 1979, Krishna *et al.,*1982 and Manjunath and Bagyaraj, 1984)[8-10]. Due to their unique capacity

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to increase nutrient uptake and water transport, they are frequently used in sustainable agriculture (Koide and Mosse, 2004)[11].

Cajanus cajan commonly known as Arhar is one of the major protein rich pulse crops grown almost every where in India. It is also used as a green manure and fodder for cattle. In comparison with rest part of India, the rate of production of Arhar is very less in Assam particularly in Barak valley. There are possibilities of increasing the rate of production of crop by the application of AMF. There fore, an attempt has been made in the present investigation to study the influences of AM fungi on the growth and phosphorus contents of *Cajanus cajan* seedlings.

MATERIAL AND METHODS

The experiment was carried out in the Department of Life science, Assam University which is situated in the Barak valley of southern Assam. Three AM fungal species namely *Glomus fasciculatum, G. aggregatum* and *Acaulospora scrobiculata* were isolated separately from the rhizospheric soil of *Cajanus cajan* by wet sieving and decanting technique (Gerdemann and Nicolson, 1963)[12]. Three AM species were maintained separately on the roots of *Zea mays* by single spore culture technique. The plants were allowed to grow for three months after which roots were severed and the substrate containing root fragments, Spores and mycelium were collected and air-dried and used as unoculum.

Healthy seeds of *Cajanus cajan* were surface sterilized with 0.2% mercuric chloride for 3 minutes and were washed 5-6 times with sterile distilled water. 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 polythene bags containing 2Kg oven sterilized mixture of sand and soil in the ratio 1:1. Seedlings were inoculated with mycorrhizal inoculum of different inoculum potential levels (½ IP, 1IP, 2IP and 4IP) of each of the three AM fungi separately. An initial inoculum potential of 13000(1IP) infective propagules per polythene bag determined by MPN (Most probable number) method (Porter, 1979)[13] was used. Five replica of each treatment were prepared.

After 120 days of transplantation, the growth of seedlings in terms of plant height and number of leaves were measured. The roots were properly washed to remove adhering soil particles. Total dry weight of the plant was determined by drying the plants in oven at 70°C for 72 hours. One g of root from every plant was separated before drying in the oven for assessment of root colonization and final dry weight was proportionally adjusted. For assessment of root colonization, root segments were washed in tap water, suitably processed and stained (Phillips and Hayman, 1970)[14]. Evaluation of colonization was done by Grid line intersect method (Giovannetti and Mosse, 1980)[15] and percentage of root colonization was calculated by the following formula

$$\%\ \text{Root Colonization} = \frac{\text{No. of segments colonized with VAM}}{\text{Total no. of segments observed}} \times 100$$

Phosphorus contents of the plant ash were determined following the method proposed by Jackson (1973)[16].

RESULTS AND DISCUSSION

Assessment of growth parameters in terms of plant height, number of leaves and total dry weight are presented in the Table 1. The results revealed that the plants inoculated with AM fungi showed significant increase in growth in terms of plant height, number of leaves, shoot fresh and dry weight, root fresh and dry weight and total dry weight in comparison with those of un inoculated plants. The over all growth of mycorrhizal plants was better as compared to the un inoculated plants. However, the response of plants varied with different AM fungi. Maximum plant growth was recorded in the plants inoculated with G.fasciculatum followed by G.aggregatum. Inoculation with G.fasciculatum enhanced the total dry weight by 72 % over uninoculated plants at 4(IP) level.

The results also revealed that seedlings varied in their response to different inoculum potential levels of different AM fungi. Growth parameters showed an increasing trend with the increase in inoculum potential levels and maximum growth response was recorded at an

Table 1. Effects of three AMF on the plant height, number of leaves per plant, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and percent increase in total dry weight of *Cajanus cajan* L. at different inoculum potential (IP) levels (Data are mean value of the five replicates)

Name of Am Fungi	Inoculum Potential Level (IP)	Plant Height (Cm)	No. of Leaves Per Plant	Shoot Fresh Weight (G/ Plant)	Root Fresh Weight (G/ Plant)	Shoot Dry Weight (G/ Plant)	Root Dry Wetght (G/ Plant)	Total Dry Weight (G/ Plant)	Increase in Total Dry total Weight (%)
Glomus fasciculatum	½	72.12	18.21	7.89	4.35	3.95	2.29	6.24	31.37
	1	78.11	21.01	9.63	4.73	4.89	2.37	7.26	52.84
	2	85.73	27.52	11.13	5.72	5.14	2.85	7.99	68.21
	4	86.01	28.80	11.45	5.69	5.35	2.82	8.17	72.00
Glomus aggregatum	½	69.57	16.67	7.15	4.37	3.21	2.28	5.49	15.58
	1	77.32	20.31	9.11	4.36	4.73	2.26	6.99	47.16
	2	83.26	24.40	10.34	5.07	5.10	2.56	7.66	61.26
	4	83.60	24.71	10.30	5.11	5.11	2.61	7.72	62.53
Acaulospora scrobiculata	½	71.03	18.20	7.63	4.39	3.37	2.21	5.58	17.47
	1	72.03	18.17	7.81	4.64	3.64	2.30	5.94	25.05
	2	74.59	19.21	8.02	4.53	4.07	2.28	6.35	33.68
	4	77.17	20.06	8.87	4.51	4.51	2.33	6.84	44.00
Uninoculated plants	0	69.83	15.4	7.03	3.56	2.95	1.80	4.75	-

inoculum potential level of 4(IP).

Root colonization increased with the increase in inoculum potential level. All the AM fungi varied significantly in colonizing the roots. Root colonization (%) by different AM fungi varied from 73% to 88% in the present study.

The plants inoculated with all the three AM fungi exhibited higher phosphorus contents as compared to un inoculated plants. Phosphorus content was highest in the plants inoculated with Glomus fasciculatum followed by *G. aggregatum*. Maximum phosphorus was recorded at 4(IP) level in both *G. fasciculatum* and *G. aggregatum* inoculated plants.

It is evident from the results that Arbuscular Mycorrhizal fungi improved the growth of the test plants in terms of plant height, leaf number and total fry weight of the plants. There are well-documented evidences that AM fungi improve the growth of the crop plants (Katiyar *et al.*,1994, Rao *et al.*,1995, Rajan, Reddy and Bagyaraj, 2000)[17-19] which support our present findings.

Root colonization by mycorrhizal fungi increased with the increase in inoculum potential level. Devi and Sitaramaiah (2001)[20] reported that AM fungal root colonization of Black gram increased with the increase in inoculum potential. The reports are in agreement with the findings of the present study.

The results of present investigation revealed that there was an increase in the phosphorus uptake by inoculated plants as compared to un inoculated plants. Good many numbers of reports are available regarding the increased uptake of phosphorus by AM fungi (Tinker, 1978, Koide and Schriener, 1992, Mc Arthur and Knowles, 1993) [21-23]. Onion plants infected with Glomus fasciculatum exhibited 14-fold increase in the uptake of phosphorus than non-mycorrhizal plants (Mohan Kumar, 1985)[24]. The contribution of mycorrhiza in the uptake of phosphorus might be due to the fact that hyphae of the fungus have large surface area leading to an increase in phosphorus absorbing surface area, production of organic acid and phosphatase, which catalyze the release of phosphorus from organic complexes(Koide and Kabir, 2000, Wang *et al.*, 2004)[25-26]. It was observed that different AM fungi differed in their ability to stimulate plant growth. Smith et al. (2003)[27] have shown that effective ness of fungi as symbionts and the plant growth response and phosphorus uptake varied significantly in different AM fungal association. Similar results were also reported by other workers (Kafkas and Orter, 2009)[28] which supports our findings. Such variations may be attributed to the mechanism of mycorrhizal infection and

Table 2. Effects of different AMF on Phosphorus uptake and root colonization in *Cajanus cajan* at different inoculum potential (IP) levels (Data are mean value of the five replicates)

Name of AM Fungi	Inoculum Potential Level(ip)	Root Colonization (%)	Phosphorus Content (%)
Glomus fasciculatum	½	76	1.35
	1	80	1.47
	2	85	1.99
	4	88	2.03
Glomus aggregatum	½	75	1.01
	1	78	1.11
	2	80	1.32
	4	84	1.29
Acaulospora scrobiculata	½	73	1.07
	1	73	1.09
	2	76	1.18
	4	80	1.25
Uninoculated plants	0	0	0.98

development (Sanders *et al.*, 1977)[29] or the physiological differences among different AM fungi in the rate of nutrient uptake and translocation (Gianinazzi and gianinazzi, 1983)[30].

CONCLUSION

Thus, the preliminary observations in our present study indicate the potential role of AM fungi particularly *Glomus fasciculatum* in enhancing the growth and phosphorus contents in *Cajanus cajan* L. Growing demand for food and increase in fertilizer cost have emphasized the need for exploitation of biosymbionts and therefore, if the production and application of appropriate quantity of mycorrhizal inoculum are done efficiently and economically, the commercial fertilizer can be replaced by these mycorrhizal biofertilizer.

ACKNOWLEDGEMENTS

Authors are thankful to HOD, Department of Life Science, Assam University for extending all sorts of support to undertake the investigation etc.

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