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Role of *Piriformospora indica* as biofertilizer for promoting growth and micronutrient uptake in *Dendrocalamus strictus* seedlings

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Abstract: The present study was conducted to assess the role of two mycorrhizal fungi (*Glomus fasciculatum* and *Piriformospora indica*) and two bacterial (*Azotobacter* and *Pseudomonas*) biofertilizers on growth and micronutrient uptake in bamboo seedlings. Various parameters such as shoot height, number of sprouts, root dry mass, per cent root colonization and micronutrient content were studied. The application of *P. indica* resulted in maximum enhancement in shoot height and number of sprouts. However, the dual inoculation of *Pseudomonas+P. indica* resulted in maximum per cent increase in shoot height over control plants, nine months after inoculation. The inoculation of *P. indica* also resulted in increased uptake of micronutrients such as zinc, copper, manganese and iron. Dual inoculation of both bacterial cultures of *Azotobacter* and *Pseudomonas* also enhanced uptake of zinc, manganese and copper, but to a lesser extent.

Key words: Biofertilizer, micronutrient, PGPR, AMF, *Piriformospora indica*

INTRODUCTION

Biofertilizers include a variety of microbial forms such as bacteria, fungi and actinomycetes, that work as nitrogen fixers, P-solubilizers, P-mobilizers and plant growth promoters. Phosphate mobilizers such as mycorrhizal forms particularly arbuscular mycorrhizal fungi (AMF) help in mineral nutrient acquisition that helps in increasing plant growth. However, the problems associated with mass production of AMF inoculum due to obligate biotrophy has deluged the benefits accorded by this fungal biofertilizer. This calls for the discovery of cultivable alternative P-mobilizing fungal forms. The recently identified hymenomycetous basidiomycete, *Piriformospora indica* has been found to have these properties (Varma *et al.*, 1999). This axenically cultivable phytopromotional endosymbiotic fungus has a broad host spectrum and

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could mimic the capabilities of arbuscular mycorrhizal fungi (Singh *et al.*, 2000). It mobilizes the insoluble phosphates and translocates the phosphorus to the host in an energy-dependent process. The other common soil rhizospheric microflora includes the plant growth promoting rhizobacteria (PGPR) that exhibit growth promotion and enhanced development on seed/soil/seedling inoculations. Being at the same rhizospheric zone, AMF and PGPRs exhibit intricate microbial interactions. Thus, in some instances the dual inoculation of these two microbes into plants results in improved growth of inoculated plants over the control. The present study was undertaken to compare and ascertain growth promotion by single and dual inoculation of PGPRs (*Azotobacter, Pseudomonas*), AMF and *P. indica.*

MATERIALS AND METHODS

The experiment was conducted on 45-days-old *Dendrocalamus strictus* seedlings raised at Department of Agronomy, Punjab Agricultural University, Ludhiana. Two bacterial cultures (*Azotobacter* and *Pseudomonas*) and two mycorrhizae (*Glomus fasciculatum* and *P. indica*) were used in the present study. The cultures were grown in suitable media on a rotary shaker (Potato dextrose broth for *P. indica,* Jensen's broth for *Azotobacter* and King's B broth for *Pseudomonas*). All the inoculations were performed at the time of planting the seedlings. Seedlings were inoculated with *P. indica* and *G. fasciculatum* at the rate of 100 and 50 spores per polythene bag containing 1.5 kg of soil. The inoculum was given in the vicinity of the seedling roots. Both the bacterial cultures were inoculated at the rate of $5x10^7$ cells per polythene bag. The experiment was laid out by using 20 seedlings per treatment in four replications. Initial data of plant height were recorded at the time of planting, and 3 and 9 months after inoculation. Different parameters such as shoot length, root dry weight, number of sprouts after three and nine months from inoculation were recorded. Root dry weight was recorded by drying the roots in an oven at 70°C for 2 days after thorough washing with tap water and 0.1 per cent HCl. Shoot length was measured with the help of a scale at two intervals of time (3 and 9 months) while root dry weight (g/plant) was determined 9 months after inoculation. The per cent root mycorrhizal colonization was studied microscopically by staining the roots (Philips and Hayman, 1970). Uptake of different micronutrients, zinc, manganese, copper and iron was studied by atomic absorption spectroscopy (Piper, 1960).

RESULTS AND DISCUSSION

All the bioinoculants exhibited growth promoting activity; however, *P. indica* followed by dual inoculation with *Azotobacter* and *Pseudomonas* recorded significant increase in shoot length over uninoculated control seedlings three months after inoculation. Similarly shoot height increased significantly over control by single and dual inoculations except for the *Pseudomonas* treatment 9 months after inoculation (Table 1). The number of sprouts was also observed to increase significantly over control for

Table 1. Effect of different biofertilizers on shoot height, number of sprouts and root weight after different time intervals

Sl. No.	Treatments	Shoot height (cm)			Number of sprouts		Root dry wt. (g/plant)
		at planting	after 3 months	after 9 months	after 3 months	after 9 months	
1	Control	18.24	19.14	30.03	1.61	2.70	13.05
2	*P. indica*	20.72	25.88	44.21	2.40	4.25	25.51
3	AMF	18.35	20.09	39.48	2.14	3.51	27.69
4	*Azotobacter*	19.44	20.50	38.53	1.65	3.55	20.80
5	*Pseudomonas*	18.49	19.21	30.22	1.60	2.73	15.70
6	*P. indica+Azotobacter*	18.90	19.95	38.27	1.64	3.39	16.30
7	*P. indica+Pseudomonas*	18.18	19.98	42.44	1.95	3.31	18.97
8	AMF+*Azotobacter*	18.74	20.21	36.26	1.78	3.23	31.50
9	AMF+*Pseudomonas*	19.85	20.99	42.82	2.00	3.34	24.01
10	*Azotobacter+Pseudomonas*	21.23	23.40	40.03	2.36	3.06	23.66
	CD (5%)	-	1.97	7.82	0.30	0.70	4.94

all the treatments except *Azotobacter* alone, *Pseudomonas* alone and dual inoculation with *P. indica+Azotobacter*. Maximum percentage increase over control in shoot height was recorded for *P. indica* and *Pseudomonas* (133.4%) followed by dual inoculation treatment of *G. fasciculatum* and *Pseudomonas* (115.7%) after 9 months from the date of transplantation which is at par with *G. fasciculatum* (115.1%) and *P. indica* (113.3%) (Fig. 1). Thus the present study indicated that dual inoculation of *P. indica+ Pseudomonas* resulted in maximum per cent increase in shoot height over uninoculated control. It was also observed that *Pseudomonas* along with mycorrhizal fungi (*P. indica* and *G. fasciculatum*) resulted in higher root length indicating a good combination of root fungi and PGPR.

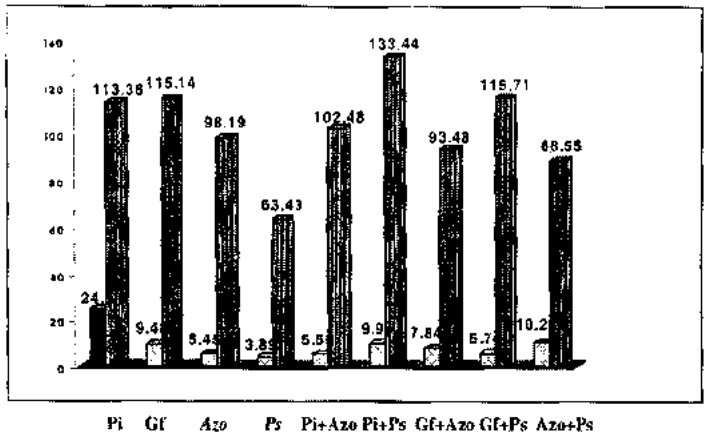

Figure 1. Per cent increase in shoot height of bamboo seedlings after 3 and 9 months of treatment.
Pi: *P. indica*, Gf: *G. fasciculatum*, Azo: *Azotobacter*, Ps: *Pseudomonas*

The root dry weight was observed to be maximum for the dual inoculation treatment of *G. fasciculatum+Azotobacter* followed by the treatment with *G. fasciculatum* alone and *P. indica* alone. All the biofertilizers led to significant increase in root dry weight over control except in the treatment with *Pseudomonas* alone and *P. indica+Azotobacter* (Table 1). The per cent increase in shoot height in *P. indica+Pseudomonas* dual treatment could be attributed to the synergistic growth promoting activity of both biofertilizers. Similarly the root biomass was observed to be maximum for the *G. fasciculatum+Azotobacter* dual treatment, thus suggesting added advantage of the dual inoculation by virtue of synergism between two biofertilizers. The mycorrhizal colonization of both *G. fasciculatum* and *P. indica* was observed as large vesicles, hyphae for the former while hyphae and characteristic pear shaped spores for the latter (Fig. 2). Similar results were reported by Muthukumar and Udaiyan (2006). They observed that combined inoculation (triple inoculation) of AMF+PSB+*Azospirillum brasilense* was the most effective in enhancing growth in bamboo both under fertilized and unfertilized conditions. Verma *et al.* (1997) and Ravikumar *et al.* (1997) reported significant increase in growth of bamboo inoculated with AM fungi.

Figure 2. Spores of *P. indica* in inoculated roots examined microscopically.

The micronutrient status of the seedlings was also studied 9 months after transplantation. There occurred a significant increase in acquisition and uptake of manganese, zinc and copper while an enormous increase in iron uptake was recorded by single inoculation with *P. indica*. This may be attributed to the presence of large body of extramatricular hyphae extending out of the root that function as accessory roots/root hairs for increased uptake of mineral nutrients. The dual inoculation with *Azotobacter+Pseudomonas* and *G. fasciculatum+Pseudomonas* also resulted in increased uptake of zinc (Table 2). Studies of Elwan (1993) show that uptake of macronutrients (P, K, Ca and Mg) and micronutrients (Fe, Mn, Zn, Cu) were highest

Table 2. Effect of different biofertilizers on micronutrient uptake in bamboo seedlings

Sl. No.	Treatments	Micronutrient status(ppm/kg)			
		Zn	Cu	Mn	Fe
1	Control	120.3	19.5	60.5	451.1
2	*P. indica*	168.08	35.4	94.8	688.1
3	AMF	139.95	22.1	67.1	470.1
4	*Azotobacter*	146.7	21.9	67.5	464.8
5	*Pseudomonas*	158.6	20.7	60.8	461.2
6	*P. indica+Azotobacter*	114.2	23.0	86.2	493.9
7	*P. indica+Pseudomonas*	135.9	19.6	82.1	451.1
8	AMF+*Azotobacter*	156.8	20.7	69.5	516.8
9	AMF+*Pseudomonas*	125.6	23.4	75.8	648.0
10	*Azotobacter+Pseudomonas*	161.56	23.5	89.3	628.6
	CD (5%)	9.51	3.19	3.58	NS

Control *P. indica* VAM Azoto Pseudo Pi+Azoto Pi+Ps V+Azoto V+Ps Azoto+Ps

Figure 3. Effect of different biofertilizer inoculations on bamboo seedlings.

in plants inoculated with *G. fasciculatum* at the recommended dose of P fertilizer. Similar studies on maize plants by Kothari *et al.* (1991) show the role of VA mycorrhiza in increased acquisition of phosphorus, zinc and copper in inoculated plants.

Thus the present study concludes that biofertilizer application can prove to be an effective technology for enhancing growth and biomass of bamboo. The various biofertilizers used in the present study helped in micronutrient acquisition and uptake from soil which in turn resulted in increasing plant height and root biomass.

REFERENCES

Elwan, I.M. 1993. Response of nutrient status of plants in calcareous soils receiving phosphorous fertilization and mycorrhiza. *Ann. Agri. Sci. Cano.* 38(2): 841-849.

Kothari, S.K., Marschner, H. and Romheld, V. 1991. Contribution of the VA mycorrhizal hyphae in acquisition of phosphorous and zinc by maize grown in a calcareous soil. *Plant and Soil* 131(2): 171-185.

Muthukumar, T. and Udaiyan, K. 2006. Growth of nursery-grown bamboo inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in two tropical soil types with and without fertilizer application. *New Forests* 31(3): 469-485.

Philips and Hayman, D. S. 1970. Improved procedures for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* 55: 158-160.

Piper, C. S. 1960. Soil and Plant Analysis. Ham Publishers, Bombay.

Ravikumar, R., Ananthakrishnan, G., Appasamy, T. and Ganapathi, A. 1997. Effect of endomycorrhizae (VAM) on bamboo seedling growth and biomass productivity. *Forest Ecol. Manage.* 98(3): 205-208.

Singh, A., Sharma, J., Rexer, K. H. and Varma, A. 2000. Plant productivity determinants beyond minerals, water and light: *Piriformospora indica*-A revolutionary plant growth promoting fungus. *Curr. Sci.* 79(11): 1548-1554.

Varma, A., Verma, S., Sudha Sahay, N., Butehorn, B. and Franken, P. 1999. *Piriformospora indica*, a cultivable plant-growth promoting root endophyte. *Applied Environmental Microbiology* 65(6): 2741-2744.

Verma, A., Govil, K.C. and Singh, Y.P. 1997. Application of biofertilizer (Arbuscular Mycorrhizal fungi) on the growth and multiplication of bamboo: An analysis for priority setting in forestry research. In: XI World Forestry Congress, 13 to 22 October 1997, Antalya, Turkey.