research article | *International Journal of Plant Sciences,* (January to June, 2010) Vol. 5 Issue 1 : 165-169

Effect of AM fungi PGPR and different soil nitrogen sources to improve growth and yield of paddy (cv. JAYA)

REKHA R. NAIK AND H.C. LAKSHMAN

Accepted : October, 2009

SUMMARY

Arbuscular mycorrhizal fungi and plant growth promoting *rhizobacteria* have a wide range of application in sustainable low input agricultural systems. The use of these two organisms may contribute to reducing chemical fertilizers and aids in high yield productivity. Studies were conducted in the net house in the botanical garden. There were 16 unsterilized mixed soil inoculum treatments with control. *Glomus macrocarpum*, *Azotobacter chroococcum* and *Glomus macro carpum + Azotobacter chroococcum* at four different levels of nitrogenous fertilizer. Paddy plants inoculated with *Glomus macrocarpum* attained maximum height when nitrogen was added @ 44 kg/ha. The *Azotobacter chroococcum* inoculated plants, however, require less nitrogen that is 24 kg/ha to attain height at par with *Glomus macrocarpum* inoculated plants. Dual inoculated plants irrespective of rate of nitrogen application produced maximum shoot biomass plants height, tiller number, 1000 grains weight N, P, in shoot than single inoculation. Effeminacy of AM fungi was significantly improved, when they were used along with *Azotobacter chroococcum*. The results of the present work strongly suggest that application of bioinoculants such as AMF and PGPR would enable farmers for optimizing paddy production with minimum input of inorganic fertilizers.

Key words : Arbuscular mycorrhizal fungi (AMF), Plant growth promoting bacteria (PGPR), *Glomus macrocarpum*, *Azotobacter chroococcum*

AMF (arbuscular mycorrhizal fungi) is widely distributed in agro-ecosystems (Smith and Read, 1997), forming symbiotic associations with the roots of plants. They play an important role in plant mineral nutrition and plant health (Barea *et al.*, 2002). These fungi have a wide range of application in sustainable low input agricultural systems (Schreiner and Bethlenfalvay, 1995). The use of AMF may contribute to reducing chemical fertilizer inputs and sustaining plant productivity in agriculture (McGonigle, 1988).

The contribution of PGPR (plant growth promoting rhizobacteria) in phytostimulation, phytoremediation, and biofertilization is well documented (Barea, 2000). *Azotobacter is* regarded as a broad-spectrum inoculant as it could be used for inoculating wide variety of crops such as wheat, rice, sorghum, barley, potato, sugarbeet, cotton, maize, etc. (Rai and Gaur, 1982).

The present study, therefore, was conducted under net house conditions using unsterilized soils, to test the effectiveness of the introduced AMF and plant growth promoting rhizobacteria in the presence of their indigenous counterparts on paddy grown at different soil nitrogen sources.

Correspondence to:
REKHA R. NAIK, Department of Post Graduate Studies in Botany (Microbiology Lab), Karnataka University, DHARWAD (KARNATAKA) INDIA
Authors' affiliations:
H.C. LAKSHMAN, Department of Post Graduate Studies in Botany (Microbiology Lab), Karnataka University, DHARWAD (KARNATAKA) INDIA

MATERIALS AND METHODS

Soil:

Test soil was collected from rice fields located near Karwara South Canara district of Karnataka. It contained 0.037% phosphorus (total), 0.109% nitrogen (total), and 1.3 ppm potassium with pH 5.70, and moisture content of 38%. Earthen pots of 20 cm diameter with a drainage hole were filled with approximately 4 kg of soil.

Experimental design and treatments:

The pot experiment was conducted in the net house in the botanical garden, Department of Botany, Karnatak University, Dharwad. The (Completely Randomized Block) design was used for the experiment. There were 16 soil treatments with control, *Glomus* only, *Azotobacter chroococcum* only, and *Glomus macrocarpum + Azotobacter chroococcum* only, at four different levels of nitrogenous fertilizer; that is, zero kg/ha, 24 kg/ha, 48 kg/ha-and 144 kg/ha. These 16 soil treatments were replicated three times. Nitrogen was applied in three splitted doses. The first dose, consisting of 1/3 the normal dose, was applied before transplantation; the second 1/3 at the time of tillering; arid the last 1/3 at the panicle initiation phase.

Collection of seeds and raising seedlings:

Paddy (*Oryza sativa* L. cv. JAYA) seeds were collected from the Department of Agriculture, University Dharwad. Seeds were surface sterilized with 2% sodium hypochlorite. They were sown on sterilized sand on trays after germination. When the seedlings were three weeks old, three seedlings were transplanted into pots with different treatments.

Bioinoculants used:

The microbial inocula used were *Glomus macrocarpum* and *Azotobacter chroococcum. Glomus macrocarpum* was isolated from different rice rhizospheres. The mycorrhizal inoculum consisting of spores, soil, and infected root fragments were obtained from the pot cultures of *Chloris gayana* Kunth. as the host plant, each pot received an inoculum of 10 g at 2 cm below the soil surface near the root system. The non-mycorrhizal pots received the same quantity of autoclaved inoculum.

Azotobacter chroococcum, was isolated from the rice rhizospriere using Ashby's medium and maintained *on* agar medium as *Azotobacter chroococcum* was enriched on nutrient media. After 24 hours, the cells were collected by centrifugation at 2000 rpm for 20 minutes and suspended in % strength Ringer's solution so as to get 5x105 cells ml"l by using haemocytometer. Each *Azotobacter chroococcum* treated pot obtained 3 ml of this cell suspension.

Measurement:

Plant height and tiller number were recorded thrice at an interval of 30 days after 1 month of transplantation. Ear number and 1000-grain weight were recorded after harvesting. Shoot and root dry weights were recorded after drying the plants in an oven at 70^0C for 48 hours and then cooling them in a dessicator. Spore count (50 g/ ads [amended soil]) was estimated by the wet sieving and decanting method (Gerdemann and Nicolson, 1963) and the percentage of root length colonized by AMF was estimated by examining stained samples (Koske and Gremma, 1989) microscopically (Brundrett *et al.*, 1984).

Determination of plant nutrient concentration:

The shoot phosphorus (P%) and nitrogen content (N%) of the plants were determined by using the ascorbic acid procedure as described in the *Laboratory methods of soil and plant analysis: a working manual* (Okalebo *et al.*, 1993) and Indophenol Blue Method (Allen, 1974), respectively, after an acid digestion treatment.

Statistical analysis:

Results were subjected to two-way analysis of variance and the significance was determined according to Duncan's Multiple Range Test (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Paddy plants varied in their response to inoculation with *Glomus macrocarpum, Azotobacter chroococcum,* and their combinations. Generally, inoculated paddy plants had greater growth compared to uninoculated controls (Table 1). Paddy plants inoculated with *Glomus macrocarpum* attained maximum height when nitrogen was added at 48 kg/ha. The *Azotobacter chroococcum* inoculated plants, however, required less nitrogen, that is, nitrogen at 24 kg/ha to attain height at par with *Glomus macrocarpum* inoculated plants. The dually inoculated plants attained maximum height when soil was fertilized with nitrogen at 48 kg/ha (Table 1).

Dual inoculated plants irrespective of rate of nitrogen application produced maximum shoot biomass. Inoculation of paddy plants with *Azotobacter chroococcum* either alone or in combination with *Glomus macrocarpum* produced maximum shoot biomass. Increasing soil nitrogen levels had a negative impact on shoot biomass in both inoculated and uninoculated plants. Efficiency of mycorrhizal fungi was significantly improved when they were used along with *Azotobacter chroococcum.*

The positive effect of microbial inoculants on tiller number, ear number, and grain yield were observed at all the levels of nitrogen-fertilization. The maximum number of tillers and ears was observed at a low level of nitrogen addition (24 kg/ha) in all microbial treatments (Table 1). More grain yield was also recorded from plants growing on low levels of nitrogen addition. Rhizobacterization of plants with *Azotobacter chroococcum* resulted in a significant increase in tiller number irrespective of whether they were mycorrhizal or non-mycorrhizal. Higher levels of soil nitrogen were inhibitory for growth and yield of rice, irrespective of the nature of microbial inoculants used (Table 1). Non significant difference was observed between *Glomus macrocarpum* and *Azotobacter chroococcum* for, their ability to induce tiller production at the same level of soil nitrogen. However, *Azotobacter chroococcum* could produce more ears than *Glomus* spp. at the highest level of nitrogen sources.

There was significant increase in shoot phosphorus content in the inoculated plants compared to uninoculated control at all the levels of nitrogen fertilization (Table 1). Plants inoculated dually with *Glomus macrocarpum* and *Azotobacter chroococcum* possessed higher shoot

Table 1 : Growth of paddy (cv. JAYA) plants as influenced by *Glomus macrocarpum* and *Azotobacter chroococcum* at different concentration of N-fertilizer sources

Microbial inoculums	N-level (kg/ha)	Height (cm)	Biomass (g)	Tiller No.	Ear no.	1000 grain weight (g)	Shoot N (%)	Shoot P (%)
Control	0	74.00 (e)	10.00 (f)	4.00 (i)	5.00	17.64 (h, i)	2.08 (e, f)	0.073 (j)
	24	82.00 (c, d, f)	15.45 (e)	5.00 (g, h, i)	10.00 (d, e)	19.07 (c, d, e)	2.70 (g, h)	0.081 (g, h)
	48	87.33 (c, d, e)	11.00 (f)	4.66 (h, i)	4.00 (h, l)	18.91 (c, d, e)	3.19 (c, d, e)	0.090 (c, d)
	144	76.00 (d, e)	8.50 (g)	3.00 (i)	3.00 (j)	13.25 (g)	3.13 (d, e)	0.076 (i)
Glomus macrocarpum	24	87.66 (a, b, c)	25.34 (b)	12.00 (b, c)	15.00 (b)	20.89 (b, c, d)	2.93 (f)	0.093 (b, c)
	48	96.33 (a)	21.64 (c)	9.00 (d, e)	10.00 (d, e)	19.80 (b, c, d, e)	3.28 (b, c, d)	0.089 (d)
	144	87.00 (b, c)	11.35 (f)	5.00 (g, h, i)	5.00 (h, i)	15.66 (f)	3.36 (a, b, c)	0.07 (h)
Azotobacter chroococcum	0	77.00 (d, e)	15.00 (e)	6.00 (f, g, h)	10.00 (d, e)	20.68 (b, c, d)	2.25 (i)	0.079 (h)
	24	91.66 (a, b)	32.67 (a)	12.00 (b, c)	16.00 (a, b)	21.19 (a, b, c)	3.08 (e, f)	0.085 (e, f)
	48	87.66 (a, b, c)	20.00 (c, d)	8.00 (d, e, f)	11.00 (c, d)	20.74 (b, c, d)	3.35 (a, b, c)	0.094 (b)
	144	78.00 (d, e)	16.00 (e)	7.00 (e, f, g)	7.00 (g)	17.20 (e, f)	3.40 (a, b)	0.082 (f, g)
Glomus macrocarpum+ Azotobacter chroococcum	0	78.00 (d, e)	18.50 (d)	7.00 (e, f, g)	12.00 (c)	22.38 (a, b)	2.62 (h)	0.092 (b, c, d)
	24	84.00 (b, c, d)	34.15 (a)	18.00 (a)	18.00 (a)	23.82 (a)	3.28 (b, c, d)	0.10 (a)
	48	90.33 (a, b, c)	27.00 (b)	14.00 (b)	16.00 (a, b)	22.50 (a, b)	3.44 (a, b)	0.103 (a)
	144	76.33 (d, e)	19.50 (d)	10.00 (c, d)	8.00 (f, g)	18.40 (d, e)	3.52 (a)	0.085 (e, f)

Means with the same letter in a column are not significantly according to DMRP, P<0.05

phosphorus content than the singly inoculated ones. The highest shoot nitrogen content was also recorded from dual inoculated plants growing on highest soil nitrogen sources.

The endogonaceous spore population was more in inoculated plants than uninoculated control plants, irrespective of the soil nitrogen concentrations (Fig. 1). Paddy plants inoculated with *Glomus macrocarpum* harboured more spore numbers than *Azotobacter chroococcum* inoculated ones. Spore numbers in the rhizosphere of dually inoculated rice plants were significantly higher than those of the singly inoculated ones. The maximum spore number was recorded from the rhizosphere of dual inoculated plants growing on 24 kg/ha of nitrogen application and the minimum spore number was recorded from uninoculated unfertilized plants.

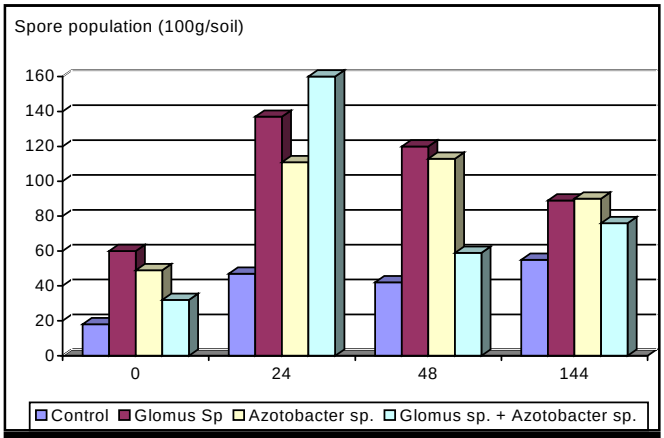

Fig. 1 : Effect of *Glmous* sp. and Azotobacter sp. on mycorrhizal spore population on paddy var. plants. Bars with same letter(s) do not differ significantly according to Duncan's Multiple Test at P<0.05

The root colonization of inoculated rice plants increased significantly from the uninoculated control (Fig. 2). *Glomus macrocarpum* only inoculated plants had more roots infected as compared to *Azotobacter chroococcum* only inoculated plants growing on all the levels of soil nitrogen concentrations. More roots were colonized when plants were inoculated with both the inoculants than with only *Glomus macrocarpum.*

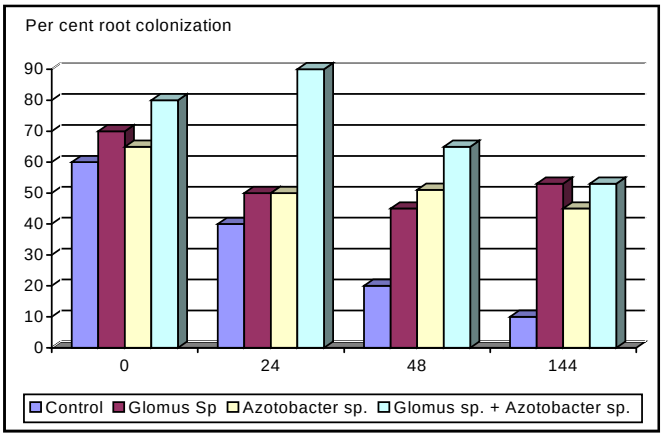

Fig. 2 : Effect of *Glmous* sp. and *Azotobacter* sp. on percentage root infection on paddy var. plants. Bars with same letter(s) do not differ significantly according to Duncan's Multiple Test at P<0.05

It has been observed that sets inoculated with *Glomus macrocarpum* and *Azotobacter chroococcum*, either alone or in combination with different levels of soil nitrogen application, positively affected crop growth over the treatments that received nitrogen only. As observed earlier (Azcon *et al.*, 2001), increasing nitrogen application had a negative effect on plant height, irrespective of whether the plants were inoculated or not. The present result indicates that inoculation of rice plants with *Glomus* sp. and, the dual inoculation of *Glomus* sp. and *Azotobacter chroococcum* enhanced the plant growth when urea was incorporated up to the recommended dose - *i.e.*, 48 kg/ha.

Paddy plants inoculated with *Glomus macrocarpum* either alone or in combination with *Azotobacter chroococcum*, grew taller than the uninoculated control plants. This is in agreement with earlier works on cotton, maize, and soybean grown on unsterilized soil (Mohan *et al.*, 1984). Rice plants for tiller production responded more to dual inoculation of AMF and only PGPRs than to either only mycorrhizal fungi or only PGPR. The present results, together with previous reports (Zambre *et al.*, 1984), confirm that association of crop yields with AMF and PGPRs enhanced the number of tillers per pot. Dhillion *et al.* (1980); Rai and Gaur (1982) and Zambre *et al.* (1984) also reported that a greater number of tillers was produced when the crop was inoculated with PGPR. Rice plants inoculated with mycorrhiza and/or rhizobacteria insignificantly increased the 1000-grain weight over uninoculated control. Reyndars and Vlassac (1982), Kundu and Gaur (1980) and Zambre *et al.* (1984) have also reported that inoculation of wheat with *Azospirillum* and *Azotobacter* increased grain yield. Such growth responses are variable and depend upon the initial fertility status of soil and the type of crop planted (Subba Rao *et al.*, 1980). The increase in growth and yield *of Azotobacter chroococcum* inoculated plants is not necessarily due to the nitrogen fixation by the added rhizobacteria (Lethbridge and Davidson, 1983), but might also be due to the growth hormones secreted by the rhizobacteria and tropical growth conditions (Wani *et al.*, 1982).

In unsterilized soil, inoculated plants showed higher spore population and higher percentage of root infection compared to the uninoculated control plants. This indicates that inoculation with mycorrhizal fungi stimulated paddy growth beyond indigenous AMF. This might be because of low indigenous AMF, which allowed effectiveness, introduced AMF to be adequately tested.

It was hypothesized that AMF would be effective only for the acquisition of slowly diffusing nutrients by plants (Lakshman and Ratageri, 2005). However, the present work demonstrates the effectiveness of mycorrhizal roots to promote nitrogen uptake, even when nitrogen, in the form of urea, is present in the soil in non-limiting amounts. This is in agreement with the recent findings of Azcon *et al.* (2001) that mycorrhizal roots promote nitrogen uptake when present in non-limiting amounts.

The addition of nitrogen at 24 kg ha^{-1} improved the growth parameters in inoculated plants. The statistically significant interaction between nitrogen addition and microbial treatments suggests that the differences in growth parameters owing to different microbial treatments were due to different rates of nitrogen application and the nitrogen response differed between inoculated and non-inoculated plants. The results of the present work strongly suggest that application of bioinoculants like AMF plus PGPR would enable farmers for optimizing paddy production with minimum input of inorganic fertilizer.

REFERENCES

Allen, S.E. (1974). *Chemical analysis of ecological materials* Blackwell scientific publication, New Delhi. pp. 1921-193.

Azcon, R., Ruiz-Rozano, J. M. and Rodriguez R. (2001). Different Contribution of arbusctilar mycorrhizal fungi to plant nitrate uptake (15 N) under increasing N supply to the soil. *Canadian J. Bot.*, **97**: 1175-1180.

Barea, J.M. (2000). Rhizosphere and mycorrhiza of field crops In: *Biological Resource Management: Connecting Science and Policy* edited by J P Toutant, E Balazs E Galante, J M Lynch, J S Schepers, D Werner and P A Werry (OECD) INRA Editions and Springer, Berlin, Heidelberg New York, pp 110-125.

Brundrett, M.C., Piche, Y. and Peterson, R L. (1984). A new method for observing the morphology of Vesicular Arbuscular mycorrhizae. *Indian J. Bot.*, **62**: 2128-2134.

Dhillion, G.J.S., Kler, D.S. and Chahal, V.P.S. (1980). Effect *of Azotobacter* inoculation along with different doses of nitrogen on growth and yield of wheat *Indian J. Agron.*, **25**: 533-535.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, **46**: 235-244.

Gomez, K.A. and Gomez, A.A. (1984). *Statistical Producers for Agricultural Research* (2nd Edn.) An International Rice Research Institute book. John Wiley and Sons, New York.

Koske, R.E. and Gremma, J.N. (1989). A modified procedure for staining roots to detect VA Mycorrhizas. *Mycological Res.*, **92** : 486-505.

Kundu, B.S. and Gaur, A.C. (1980). Establishment of nitrogen-fixing and phosphate solublizing bacteria in rhizosphere and their effect on yield and nutrient uptake of wheat crop. *Plant & Soil*, **57**:223-230.

Lakshman, H.C. and Ratageri, Ramesh, H. (2005). Dual inoculation of VA-mycorrhiza and *Rhizobium* beneficial to *Pithecblobium dulce* Roxb. *J. Microbial. Word.*, **7**(2): 163-169.

Lethbridge, G. and Davidson, M.S. (1983). Root associated nitrogen fixing bacteria and their role in the nitrogen nutrition of wheat estimated by ^{15}N isotope dilution *Soil Biol. & Biochem.*, **15** : 365-374.

Mc Gonigle, T.P. (1998). A numerical analysis of published field trials with vesicular arbuscular mycorrhizal fungi. *Functional Ecol.*, **2**: 473-478.

Mohan, R., Bagyaraj, D. J. and Manjunath, A. (1984). Response of crop plants to Vesicular-Arbuscular Mycorrhizal inoculation in five unsterile soils Karnataka. *J. Soil Biology & Ecol.*, **4** (1) : 6-12.

Okalebo, J.R., Gathua, K.W. and Woomer, P.L. (1993). Laboratory Methods of Plant and Soil Analysis: A Working Manual. Technical Bulletin No. 1, Soil Science Society, East Africa.

Rai, S. N. and Gaur, A.C. (1982). Nitrogen fixation by *Azospirillum* spp and effect of *Azospirillum lipoferum* on the yield and N-uptake of wheat crop. *Plant & Soil*, **69** : 233-238.

Reyndars, L. and Vlassak, K. (1982). Use of *Azospirillum brasilence* as biofertizer in intensive wheat cropping. *Plant & Soil*, **66**: 217-223.

Schreiner, R.P., and Bethenfalvay, G.J. (1995). Mycorrhizal interactions in sustainable agriculture. *Critical Rev. Biotechnol.*, **15**:271-287.

Smith, S.E. and Read, D.J. (1997). *Mycorrhizal Symbiosis* Academic Press, San Diego. 605 pp.

Subba Rao, N.S., Tilak, K.V.B.R., Lakshmikumari, M. and Singh, C.S. (1980). *Azospirillum* a new bacterial fertilizer. *Indian Farming*, **30**: 3-5.

Wani, S.P., Dart, P.L. and Subba Rao, R.V. (1982). Factors affecting the nitrogenase activity of sorghum and millet estimated by soil-root core assay Method National Symposium on Biological Nitrogen Fixation. IARI. New Delhi 57pp.

Zambre, M.A., Konde, B.K. and Sonar, K.R. (1984). Effect of *Azotobacter chroococcum* and *Azospirillum brasilense* inoculation under graded levels of nitrogen on growth and yield of wheat. *Plant & Soil*, **79**: 61-67.

****
*****