World Journal of Microbiology & Biotechnology 10, 381–384

Evaluation and first-year field testing of efficient vesicular arbuscular mycorrhizal fungi for inoculation of wetland rice seedlings

J. Secilia and D.J. Bagyaraj*

Grain yields of the rice cultivar 'Prakash' were improved upon inoculation with *Glomus intraradices* and *G. fasciculatum*, by 11% and 8%, respectively, compared with an uninoculated control. The results indicate that the amount of phosphate fertilizer usually applied to rice may be decreased by 50%, without affecting yield, if *G. intraradices* is inoculated.

Key words: *Glomus intraradices*, mycorrhiza, *Oryza sativa*, rice.

Many pot and field experiments show that the introduction of a new vesicular arbuscular mycorrhizal (VAM) fungus into natural soil is possible and that the introduced fungus can improve plant growth (Jeffries 1987). Increased yield in field trials after inoculation with efficient VAM fungi has been reported (Lu & Miller 1989). Because of the difficulties in producing VAM inoculum in large quantities, VAM inoculation technology can be used more efficiently in nursery-raised, transplanted crops than in direct-seeded field crops (Bagyaraj 1992). Inoculation with efficient VAM fungi can permit the amount of phosphate fertilizer given to transplanted crops to be reduced by as much as 50% without significant effect on yield (Govinda Rao *et al.* 1983; Sreeramulu & Bagyaraj 1986).

Rice is a staple food for approximately one half of the world's population yet its response to inoculation with VAM fungi is poorly understood. Improved plant growth and Zn^{2+} nutrition was reported in upland (Sharma *et al.* 1988) and wetland (Secilia & Bagyaraj 1992) rice inoculated in pot experiments with VAM fungi. In screening tests, we recently selected isolates of *Glomus intraradices*, *G. fasciculatum* and *Acaulospora* for use in inoculating wetland rice (Secilia & Bagyaraj 1992); *G. intraradices* was the most efficient fungus. The present study was undertaken to show the response of wetland rice to inoculation with these three fungi under field conditions, at three concentrations of phosphate fertilizer application.

The authors are with the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065, India. *Corresponding author.

Materials and Methods

Cultivation of Rice and Inoculum Preparation

The soil used in the experiment was a phosphate deficient [11.6 mg P kg^{-1}, determined by Bray's method (Jackson 1973)] sandy clay loam of pH 6.9, with an indigenous VAM population of 14 infective propagules g^{-1} soil, determined by the most probable number (MPN) procedure (Porter 1979). 'Prakash', a cultivar of wetland rice grown locally, was selected for use in the study. Four raised nursery beds (2 m × 1 m × 15 cm) were prepared and fertilized with the recommended level of N, P and K [1 kg N, as $(NH_4)_2SO_4$, 0.17 kg P, as super phosphate, and 0.28 kg K, as KCl, per 100 m^2]. Root pieces and soil from 3-month-old pot cultures of *Panicum maximum* Jacq. (Guinea grass) colonized by *Glomus intraradices*, *G. fasciculatum* or *Acaulospora* sp, were used at 5 kg dry wt per nursery bed and mixed thoroughly into the top 2.5 cm of soil. The control nursery bed received inoculum collected from non-mycorrhizal pots (maintained with *P. maximum* but without any mycorrhizal fungi) to introduce soil microflora other than mycorrhizal fungi. Paddy seeds were broadcast on the drained nursery bed at the rate of 80 g m^{-2}. The beds were kept moist till the seedlings attained 25 cm height and then water was let into the beds to maintain a shallow layer (approximately 4 cm) of standing water.

The experimental plot was divided into four blocks, each having 12 subplots (of 2.0 m × 1.0 m). All received N and K, at the rate of 100 kg N ha^{-1} as $(NH_4)_2SO_4$ and 50 kg K^+ ha^{-1} as KCl, as recommended for wetland rice in this region. N was applied in three split doses, 50% before transplanting, 25% 30 days after transplanting and the remaining 25% at panicle initiation stage. There were three different levels of phosphate application: no phosphate; half the recommended phosphate (i.e. 25 kg P ha^{-1}); and the recommended phosphate application (50 kg P ha^{-1}).

Phosphate was applied in two equal doses, just before transplanting and 30 days after transplanting. There were 12 treatments (four inoculation treatments each at three levels of phosphate), each replicated four times in a randomized block design.

Seedlings (28 days old) grown in nursery beds were transplanted to the experimental plots at a hill spacing of 20 × 10 cm, with three seedlings per hill and 81 hills per sub-plot. Plots were irrigated from a nearby tank and a shallow layer (5.0 cm) of water was maintained in all the plots. Five plants selected at random (using double-digit random numbers for each plot) were uprooted on days 60, 120 and 145 after sowing.

Analyses

Mycorrhizal colonization (%) of roots was determined after clearing roots with KOH and staining with Trypan Blue (Phillips & Hayman 1970). Mycorrhizal spore numbers in root-zone soil was estimated by wet sieving and decantation (Gerdemann & Nicolson 1963).

Plants were harvested 145 days after sowing, when grain filling was complete. Dry weights of shoots, roots and grain were recorded after drying to a constant weight. Plant phosphate content was determined by the vanadomolybdate phosphoric method (Jackson 1973).

The data were subjected to statistical analysis suitable for factorial randomized block design experiments (Sunderraj *et al.* 1972). Treatment means were separated by Duncan's multiple range test (Little & Hills 1978).

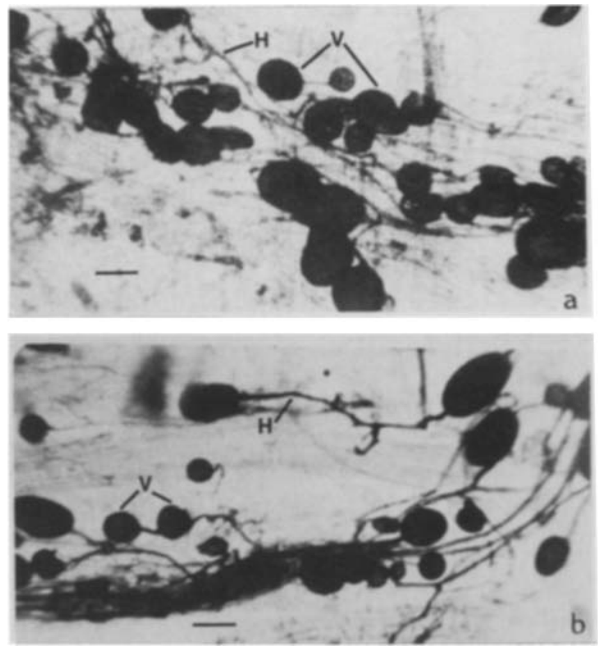

Figure 1. Colonization of the wetland rice cultivar 'Prakash' by (a) *Glomus intraradices* and (b) *G. fasciculatum*, showing vesicles (V) and hyphae (H). Marker bar = 50 μm.

Results and Discussion

Mycorrhizal spore numbers and percentage root colonization were significantly greater in inoculated plants (except for root colonization in *Acaulospora* inoculated plants) than in uninoculated plants (Table 1). Both these mycorrhizal parameters decreased when the phosphate fertilizer was increased. Mycorrhizal colonization ranged only from 17% to 44%. This confirms a previous report (Manjunath *et al.* 1981) which showed low percentage colonization in submerged rice cultivars. Mycorrhizal colonization of the wetland rice 'Prakash' by *G. intraradices* and *G. fasciculatum* is shown in Figure 1.

The three VAM fungal isolates studied differed in the efficiency with which they increased plant dry weight (Table 2). Shoot dry weight of plants inoculated with *G. intraradices* was significantly higher (27%) than in uninoculated control plants. However, the observed increase in root dry weight due to inoculation with VAM fungi was not statistically significant ($P = 0.05$).

All three VAM fungal isolates significantly increased shoot phosphate content in plants receiving no added phosphate fertilizer. Plants given the recommended amount of phosphate fertilizer also had significantly higher shoot phosphate content than uninoculated plants and plants given half the recommended phosphate. Hall *et al.* (1977) reported that application of mycorrhiza plus phosphate fertilizer at high con-

Table 1. Effect of VA mycorrhizal inoculation at varying amounts of phosphate fertilizer on the mycorrhizal spores in root-zone soil and root colonization of field-grown rice at harvest.*

Inoculum	Spore count (spores/25 ml soil)				Mycorrhizal root colonization (%)			
	P_0	$P_{½}$	P_1	Mean	P_0	$P_{½}$	P_1	Mean
None	100^{cd}	80^{d}	88^{cd}	89^{z}	24^{a}	26^{a}	22^{a}	24^{y}
Glomus fasciculatum	171^{a}	141^{ab}	72^{d}	128^{y}	39^{a}	35^{a}	31^{a}	31^{x}
Acaulospora sp.	173^{a}	117^{c}	171^{a}	154^{x}	30^{a}	25^{a}	23^{a}	26^{y}
Glomus intraradices	167^{a}	146^{ab}	163^{a}	159^{x}	42^{a}	31^{a}	27^{a}	33^{x}
Means	153^{x}	121^{y}	123^{y}		34^{x}	29^{y}	26^{y}	

*Values with common superscript letters for the interaction effects in a block, or the main effects (means), do not differ significantly according to Duncan's multiple range test at $P = 0.05$.

P_0—No phosphate; $P_{½}$—half the recommended phosphate (25 kg P ha^{-1}); P_1—recommended amount of phosphate (50 kg P ha^{-1}).

Table 2. Effect of VA mycorrhizal inoculation at varying amounts of phosphate fertilizer on root and shoot dry weight of field-grown rice at harvest.*

Inoculum	Shoot dry wt (g/plant)				Root dry wt (g/plant)			
	P_0	$P_{1/2}$	P_1	Mean	P_0	$P_{1/2}$	P_1	Mean
None	8.6^{a}	10.8^{a}	9.2^{a}	9.3^{y}	4.5^{d}	5.1^{abcd}	5.8^{ab}	5.1^{x}
Glomus fasciculatum	9.2^{a}	9.9^{a}	10.5^{a}	9.9^{y}	4.9^{bcd}	5.8^{ab}	5.6^{abc}	5.4^{x}
Acaulospora sp.	9.2^{a}	9.7^{a}	9.6^{a}	9.5^{y}	4.8^{cd}	5.2^{abcd}	5.7^{ab}	5.2^{x}
Glomus intraradices	10.1^{a}	10.1^{a}	10.0^{a}	10.5^{x}	5.9^{a}	5.3^{abcd}	5.8^{a}	5.6^{x}
Means	9.3^{x}	10.1^{x}	10.4^{x}		5.0^{x}	5.4^{x}	5.7^{x}	

*Values followed by common superscript letters for the interaction effects in a block, or the main effects (means) do not differ significantly according to Duncan's multiple range test at $P = 0.05$.
P_0—No phosphate; $P_{1/2}$—half the recommended phosphate (25 kg P ha^{-1}); P_1—recommended amount of phosphate (50 kg P ha^{-1}).

Table 3. Effect of VA mycorrhizal inoculation at varying concentrations of phosphate fertilizer on grain yield of field-grown rice.*

Inoculum	Grain yield (g/plot)			
	P_0	$P_{1/2}$	P_1	Mean
None	840^{bc}	860^{c}	877^{bc}	859^{z}
Glomus fasciculatum	872^{abc}	965^{a}	950^{ab}	929^{y}
Acaulospora sp.	848^{c}	904^{abc}	889^{bc}	880^{yz}
Glomus intraradices	904^{abc}	981^{a}	976^{a}	954^{x}
Means	891^{x}	952^{x}	923^{x}	

*Values followed by common superscript letters for the interaction effects in a block, or the main effects (means), do not differ significantly according to Duncan's multiple range test at $P = 0.05$.
P_0—No phosphate; $P_{1/2}$—half the recommended phosphate (25 kg P ha^{-1}); P_1—recommended amount of phosphate (50 kg P ha^{-1}).

centrations (> 30 kg P ha^{-1}) in a silt loam soil depressed growth of clover. In the present study, at the recommended phosphate dose (50 kg P ha^{-1}), none of the isolates depressed plant growth.

Comparing the main effects, inoculation with *G. intraradices* and *G. fasciculatum* significantly increased the grain yield over uninoculated plants by 11 and 8%, respectively, and the two fungi were statistically equivalent at all three concentrations of phosphate fertilizers used (Table 3). *Glomus intraradices* was the best fungus at increasing grain yield, with both 50% phosphate and the recommended level of phosphate. Grain yields with *G. intraradices* inoculation were 14 and 11% higher than controls at 50% and 100% phosphate, respectively. The corresponding yields with *G. fasciculatum* inoculation were 12 and 8% higher than controls respectively. The highest grain yield achieved was obtained when plants were inoculated with *G. intraradices* and provided with 50% of the normal application of phosphatic fertilizer.

The shoot and grain phosphate contents are given in Table 4. There were positive correlations between shoot dry weight and phosphate content and between grain yield and phosphate content. This indicates that the observed increases in dry weight and yield caused by inoculation with mycorrhizal fungi are mainly the result of increased uptake of phosphate by these fungi.

Grain yields of the uninoculated plants given the recommended level of phosphate fertilizer (50 kg P ha^{-1}) and the plants inoculated with VAM fungi and raised with either no

Table 4. Effect of VA mycorrhizal inoculation at varying amounts of phosphate fertilizer on shoot and root phosphate content of field grown rice.*

Inoculum	Shoot phosphate (mg/plant)				Root phosphate (mg/plant)			
	P_0	$P_{1/2}$	P_1	Mean	P_0	$P_{1/2}$	P_1	Mean
None	32^{d}	44^{c}	48^{bc}	41^{y}	51^{d}	68^{bcd}	96^{ab}	72^{x}
Glomus fasciculatum	44^{c}	53^{abc}	47^{bc}	48^{xy}	57^{cd}	88^{ab}	78^{abcd}	75^{x}
Acaulospora sp.	52^{abc}	46^{bc}	60^{a}	53^{x}	90^{ab}	61^{d}	80^{abc}	77^{x}
Glomus intraradices	55^{ab}	49^{bc}	56^{ab}	53^{x}	99^{a}	68^{bcd}	52^{cd}	73^{x}
Means	50^{y}	48^{y}	53^{x}		74^{x}	71^{x}	77^{x}	

*Values followed by common superscript letters for the interaction effects in a block, or the main effects (means), do not differ significantly according to Duncan's multiple range test at $P = 0.05$.
P_0—No phosphate; $P_{1/2}$—half the recommended phosphate (25 kg P ha^{-1}); P_1—recommended amount of phosphate (50 kg P ha^{-1}).

phosphatic fertilizer or half the recommended phosphate were statistically equivalent. This first-year field test indicates that all the phosphate fertilizer normally applied may be saved through inoculation with VAM fungi. Such a saving would, however, deplete soil phosphate completely if practised year after year and it would be safer to use 50% of the recommended phosphate fertilizer along with VAM fungal inoculation. It is also evident from the present study that VAM fungi differ in their efficiency at improving the growth of wetland rice. The investigation also indicated that *G. intraradices* was the best of the fungi tested for inoculating the wetland rice cultivar 'Prakash'. As rice is traditionally a transplanted crop, the simple, low-cost technology of applying mycorrhizal inoculum to the nursery can easily be followed by farmers.

References

Bagyaraj, D.J. 1992 Vesicular arbuscular mycorrhiza: application in agriculture. *Methods in Microbiology* **24**, 359–374.

Gerdemann, J.W. & Nicolson, T.H. 1963 Spores of mycorrhizal *Endogone* species extracted by wet sieving and decanting. *Transactions of the British Mycological Society* **46**, 235–244.

Govinda Rao, Y.S., Bagyaraj, D.J. & Rai, P.V. 1983 Selection of efficient VA mycorrhizal fungus for finger millet II. Screening under field conditions. *Zentralblatt für Mikrobiologie* **138**, 415–419.

Hall, I.R., Scott, R.S. & Johnstone P.O. 1977 Effect of vesicular-arbuscular mycorrhizas on response of grass lands 'Huio' and 'Tamar white' clovers to phosphorus. *New Zealand Journal of Agricultural Research* **20**, 349–355.

Jackson, M.L. 1973 *Soil Chemical Analysis*. New Delhi: Prentice Hall.

Jeffries, P. 1987 Use of mycorrhizae in agriculture. *Critical Reviews in Biotechnology* **5**, 319–357.

Little, T.M. & Hills, F.J. 1978 *Agricultural Experimental Design and Analysis*. New York: John Wiley & Sons.

Lu, S. & Miller, M.H. 1989 The role of VA mycorrhiza in the absorption of P and Zn by maize in field and growth chamber experiments. *Canadian Journal of Soil Science* **69**, 97–109.

Manjunath, A., Mohan, R., Raj, J. & Bagyaraj, D.J. 1981 Vesicular-arbuscular mycorrhiza in cultivation of rice. *Journal of Soil Biology and Ecology* **1**, 1–4.

Phillips, J.M. & Hayman, D.S. 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungus for rapid assessment of infection. *Transactions of the British Mycological Society* **55**, 158–161.

Porter, W.M. 1979 The most probable number method for enumerating infective propagules of vesicular arbuscular mycorrhizal fungi in soil. *Australian Journal of Soil Research* **17**, 515–519.

Secilia, J. & Bagyaraj, D.J. 1992 Selection of an efficient vesicular-arbuscular mycorrhizal fungus for wetland rice. *Biology and Fertility of Soils* **13**, 108–111.

Sharma, A.K., Singh, R. & Singh, V.S. 1988 Effect of vesicular-arbuscular mycorrhiza in uptake of phosphorus and zinc in rice (*Oryza sativa* L.). *Current Science* **57**, 901–903.

Sreeramulu, K.R. & Bagyaraj, D.J. 1986 Field response of chilli to VA mycorrhiza on black clayey soil. *Plant and Soil* **93**, 299–302.

Sunderraj, N., Nagaraj, S., Venkatarama, M.N. & Jagannath, M.K. 1972 *Design and Analysis of Field Experiments*. Bangalore: University of Agricultural Sciences.

(Received in revised form 11 January 1994; accepted 18 January 1994)