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Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.)

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Summary. We tested the response of the wetland rice cultivar Prakash to inoculation with ten vesicular-arbucular mycorrhizal (VAM) fungi (three selected from the first screening and seven isolated from local paddy fields) in a pot experiment under flooded conditions, in order to select the most efficient mycorrhizal fungi to inoculate the rice nursery. A sandy clay loam soil was used as the substrate, fertilized with the recommended N and K levels (100 kg N ha^{-1} as ammonium sulphate and 50 kg K ha^{-1} as muriate of potash) and half the recommended level of P (25 kg ha^{-1} as super phosphate). The inoculation was made into dry nursery beds and the beds were flooded when the seedlings were about 25 cm high, in 15 days. Twenty-eight-day old seedlings were transferred to pots filled with well puddled soil flooded with 5 cm of standing water. Based on the increase in grain yield and total biomass, *Glomus intraradices* and *Acaulospora* sp. were considered efficient and suitable for inoculation into rice nurseries.

Key words: *Oryza sativa* L. – Vesicular-arbuscular mycorrhiza – *Glomus* spp. – *Acaulospora* spp. – Wetland rice – Rice inoculation

The beneficial effects of inoculating crop plants with VAM fungi are becoming widely appreciated, mainly because these fungi can improve P nutrition under certain conditions (Jeffries 1987). Recent work has shown improved plant growth and improved P and Zn nutrition of wetland rice under pot culture conditions following inoculation with VAM fungi isolated from different non-paddy soils (Sharma et al. 1988; Secilia and Bagyaraj 1992).

Different species of VAM fungi vary widely in their ability to stimulate crop production (Reena and Bagyaraj 1990), even in case of wetland rice grown under flooded conditions (Secilia and Bagyaraj 1992). In a preliminary trial we found that VAM fungi were promising for inoculating wetland rice (Secilia and Bagyaraj 1992). The present study is part of a continuing program aimed at selecting an efficient inoculant VAM fungus for rice. We report here the results of an investigation on seven local fungi isolated from different paddy fields plus the three best fungi selected from a preliminary screening trial.

Materials and methods

The soil used in the experiment was a P-deficient (11.6 mg kg^{-1}, determined by Bray's method) sandy clay loam of pH 6.9 with an indigenous endomycorrhizal population of 14 infective propagules g^{-1} soil. The physicochemical properties of the soil used in the study are given in Table 1. Prakash, a cultivar of wetland rice grown around Bangalore, was

Table 1. Some physicochemical properties of the sandy clay loam soil used in the pot experiment

Characteristics	Magnitude	Method of determination
Mechanical analysis		
Coarse sand	27.6%	
Fine sand	24.5%	Bouyoucos hydrometer (Piper 1966)
Salt	13.4%	
Clay	34.5%	
Physicochemical analysis		
pH	6.9	Beckman pH meter (Piper 1966)
Organic matter	1.23%	Walkley and Black method (Jackson 1973)
Available P	11.6 ppm	Bray method (Jackson 1973)
Available K	55.7 ppm	Flame photometer method (Jackson 1973)
EC at 25 °C	0.87	Conductivity meter (Jackson 1973)
Available Zn	145 ppm	Atomic absorption spectrophotometer (Isaac and Kerber 1971)

EC, electrical conductivity

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used in the present study. Seven VAM fungi isolated from paddy fields in different regions of Karnataka state, by wet sieving and decantation (Gerdemann and Nicolson 1963), were brought into pot culture by the funnel technique. These seven fungi plus the three fungi selected as the best from the first trial reported earlier (listed in Table 2) were used in the present experiment. Root pieces and soil from pot cultures of *Panicum maximum* Jacq. colonized with the different test fungi were used as inocula. According to the most probable number of infective propagules in each pot culture (Porter 1979), 50000 infective propagules were added to a nursery box of each fungi.

A dry nursery bed was prepared in 12 boxes (48 cm long×25 cm broad×21 cm deep) of 8 kg capacity. Recommended levels N and K (1 kg N as ammonium sulphate and 0.23 kg K as muriate of potash per 100 m^2) were applied to all the boxes. Half the recommended level of P was applied to 11 boxes and the full recommended level was applied to one control box (0.09 kg and 0.17 kg P, respectively, per 100 m^2 as superphosphate). The mycorrhizal inoculum was spread over the puddled, water-drained, unsterile soil which served as the nursery bed and mixed thoroughly into the top 2.5 cm of soil. The two control boxes (one with half P and the other with full P) were supplied with inoculum collected from non-mycorrhizal pots (maintained with *Panicum maximum* without any mycorrhizal fungi) to introduce soil microflora other than mycorrhizal fungi. Pre-germinated rice seeds were sown on the drained nursery bed. The beds were kept moist until the seedlings were about 25 cm high (15 days) and then a shallow layer (5 cm) of water was allowed to stand on the soil in the nursery trays. The mycorrhizal and non-mycorrhizal seedlings were removed on the 28th day and transplanted to plastic pots of 9 kg capacity. The soil in these pots was puddled thoroughly and fertilizer was applied at the rate of 100 kg N as ammonium sulphate, 25 kg P (half dose) as superphosphate, and 50 kg K as muriate of potash ha^{-1} to 11 treatments before transplanting. One control was supplied with 100 kg N, 50 kg P, and 50 kg K ha^{-1}, which is the recommended level of fertilizer for wetland rice in this region. Two hills, each with three seedlings, were maintained in each pot. The pots were maintained with 5 cm of standing water on the soil.

The plants were harvested 145 days after sowing, when grain filling was complete. Dry weights of shoots, roots, and grain were recorded after drying the plant materials to a constant weight. Dried root and shoot samples of each plant were used for nutrient analyses. The plant P content was determined by the vanadomolybdate phosphoric yellow colour method, as outlined by Jackson (1973). Mycorrhizal spore numbers in the soil were estimated by the wet sieving and decantation method described by Gerdemann and Nicolson (1963). The percentage mycorrhizal colonization of roots was determined by clearing the roots with KOH and staining them with trypan blue (Phillips and Hayman 1970).

The data were subjected to statistical analyses suitable for a random complete-block design. The treatment means were separated by Duncan's multiple range test (Little and Hill 1978).

Results

The rice showed varied responses to inoculation with the different VAM fungi. By harvest-time, the plants inoculated with *G. intraradices* Po were 15.2% higher than the uninoculated controls, although this increase was not statistically significant (Table 2). Nine out of the 10 test fungi significantly increased the number of plant tillers, the increase ranging from 10.1% to 20.3%

The increases in shoot biomass following inoculation ranged from 49.5% to 174%, and all were significant (Table 3). The highest increase in shoot weight was found in plants inoculated with *G. fasciculatum* Ri and *G. intraradices*. The increase in the root weight induced by different VAM fungi ranged from 64.05% to 153.6% (Table 3). All the mycorrhizal treatments gave significantly higher grain weights than the uninoculated controls. The highest grain weight increase was obtained in plants inoculated with *G. intraradices* (214%). All the mycorrhizal treatments recorded a significantly higher total biomass (root+shoot+grain) than the uninoculated controls with half the recommended P fertilizer. The maximum increases were recorded in plants treated with *G. intraradices* (169.4%), *G. fasciculatum* Ri (165%), and *Acaulospora* sp. (152.2%).

Inoculation with VAM fungi significantly increased P concentrations in the rice (Table 4). The shoot P concentration was highest in plants treated with *G. intraradices* (0.44%; Table 4), while the maximum increase in root P concentration was recorded in plants treated with *G. intraradices* (0.52%). Plant (shoot+root) P concentrations varied from 0.73% to 0.93% among the different mycorrhizal treatments. In the uninoculated controls with half the recommended level of P fertilizer, the plant P concentration was 0.55%. The increase was highest in plants treated with *G. intraradices* (0.93%); these plants had significantly higher P concentrations even compared to the control plants supplied with the full P fertilizer. The difference in grain P concentrations among different treatments was not statistically significant.

The percentage mycorrhizal root colonization (Table 5) was significantly greater in all the mycorrhizal treatments than in the uninoculated controls. The greatest root colonization was found in plants inoculated with *G. intraradices* Po. Spore counts were highest in plants inoculated with *G. intraradices*.

Discussion

The mycorrhizal enhancement of plant growth is generally attributed to increased nutrient uptake, especially P

Table 2. Effect of different vesicular-arbuscular mycorrhizal fungi on plant height, number of tillers and number of panicles in rice plants

Treatments	Plant height (cm)	Tillers (no. $plant^{-1}$)	Panicles (no. $plant^{-1}$)
Uninoculated (half P)	88.2	2.4c	2.0c
Uninoculated (full P)	102.8	6.3ab	3.9b
Acaulospora sp. (IC)	99.5	7.2a	3.7b
A. foveata (Mn)	93.9	4.7bc	4.5ab
A. scrobiculata (Hb)	94.4	5.4ab	4.2ab
Glomus aggregatum (Mg)	92.6	5.0ab	3.4bc
G. claroideum (Cu)	98.0	6.8ab	5.0ab
G. etunicatum (Kp)	78.8	4.8b	3.7b
G. fasciculatum (Ri)	102.3	7.2a	3.8b
G. mosseae (In)	97.8	5.9ab	5.0ab
G. intraradices (Po)	101.6	6.0ab	5.8a
G. versiforme (Si)	102.2	6.2ab	3.8b

Values without common letters differ significantly according to Duncan's multiple range test at $P = 0.05$. Half p, 25 kg ha^{-1}; full p, 50 kg ha^{-1}; IC, ICRISAT, India; In, Invermay Research Station, New Zealand; Mn, Regional Research Station, Mandya; Mg, Regional Research Station, Mangalore; Cu, Central Rice Research Institute, Cuttack; Hb, Hebbal, Bangalore; Kp, Krishnarajapuram, Bangalore; Po, Ponnampet, Coorg; Si, Regional Research Station, Siruguppa; Ri, Riverside, USA

Table 3. Effect of different vesicular-arbuscular mycorrhizal fungi on shoot, root, grain and total biomass of rice plants

Treatments	Dry weight (g plant^{-1})			
	Shoot	Root	Grain	Total
Uninoculated (half P)	5.6e	3.1e	3.3f	11.9e
Uninoculated (full P)	14.5ab	7.4ab	8.4bcd	30.0abc
Acaulospora sp. (IC)	12.6bc	6.1bcd	9.7ab	30.0abc
A. foveata (Mn)	8.4d	3.5e	4.8e	16.7d
A. scrobiculata (Hb)	12.3c	6.8abc	8.0bcd	27.3b
Glomus aggregatum (Mg)	11.4c	6.3bcd	8.5bc	26.2c
G. claroideum (Cu)	14.6ab	5.0d	7.8cd	27.4bc
G. etunicatum (Kp)	13.1bc	6.8abc	6.8d	25.5c
G. fasciculatum (Ri)	15.5a	6.9abc	8.4bcd	31.6ab
G. mosseae (In)	12.3c	5.8cd	8.0bcd	25.9c
G. intraradices (Po)	15.4a	7.8a	10.1a	32.1a
G. versiforme (Si)	11.9c	6.9abc	8.4bcd	27.4bc

See footnotes to Table 2

Table 4. Effect of different vesicular-arbuscular mycorrhizal fungi on shoot, root, grain and total P concentration of rice

Treatments	P concentration (%)		
	Shoot	Root	Plant (shoot + root)
Uninoculated (half P)	0.21e	0.35d	0.55d
Uninoculated (full P)	0.28de	0.33d	0.62cd
Acaulospora sp. (IC)	0.40ab	0.49ab	0.81abc
A. foveata (Mn)	0.32bcd	0.46abc	0.76abc
A. scrobiculata (Hb)	0.32bcd	0.36cd	0.82ab
Glomus aggregatum (Mg)	0.27de	0.41bcd	0.73bcd
G. claroideum (Cu)	0.38ab	0.44abcd	0.75abc
G. etunicatum (Kp)	0.30d	0.43abcd	0.75abc
G. fasciculatum (Ri)	0.38abc	0.41bcd	0.75abc
G. mosseae (In)	0.42a	0.40bcd	0.82ab
G. intraradices (Po)	0.44a	0.52a	0.93a
G. versiforme (Si)	0.39abc	0.37cd	0.79abc

See footnotes to Table 2

Table 5. Effect of different vesicular-arbuscular mycorrhizal fungi on mycorrhizal root colonization and spore counts in soil

Treatments	Mycorrhizal root colonization (%)	Spore counts in 25 ml soil (no.)
Uninoculated (half P)	15.2 (22.9)f	45.0d
Uninoculated (full P)	15.0 (22.8)f	72.4d
Acaulospora sp. (IC)	31.0 (33.8)b	151.6ab
A. foveata (Mn)	19.8 (26.3)e	110.8c
A. scrobiculata (Hb)	22.2 (28.1)de	126.0bc
Glomus aggregatum (Mg)	26.8 (31.1)bcd	153.6ab
G. claroideum (Cu)	26.2 (30.7)bcd	140.6abc
G. etunicatum (Kp)	24.8 (29.8)cd	131.4abc
G. fasciculatum (Ri)	26.8 (31.1)bcd	154.4ab
G. mosseae (In)	28.8 (32.4)bc	154.0ab
G. intraradices (Po)	39.4 (38.9)a	167.2a
G. versiforme (Si)	24.8 (29.9)cd	128.8dc

Values in parentheses are arc-sine transformed; for other explanations, see footnotes to Table 2

(Jeffries 1987). Mycorrhizal benefits have been reported for many cereals, including pearl millet, wheat, and maize (Rao et al. 1983; Lu and Miller 1989). There are also some reports of a positive response by upland rice plants to VAM inoculation (Gangopadhyay and Das 1984; Dash et al. 1987; Iqbal et al. 1978). Though wetland rice has previously been considered non-mycorrhizal, a positive response to VAM inoculation has recently been observed (Sharma et al. 1988). The present study, in which several VAM fungi were used to inoculate rice plants, has shown significant increases in the number of plant tillers and panicles, plant biomass, plant P contents, and grain yields following inoculation.

Of the VAM fungi used in the present trial *G. intraradices* Po gave the best performance in all the growth parameters studied. The next best fungus was *Acaulospora* sp. IC. The increase in grain yield following inoculation with these fungi was nearly threefold compared to the uninoculated controls. These results show that selected species of VAM fungi can survive under flooded conditions and can colonize rice roots to increase the uptake of P and plant growth. The best fungus, *G. intraradices*, was isolated from Ponnampet, Coorg, where rice is grown under flooded conditions throughout the year. This strain may have adapted to wetland conditions and thus provided the maximum response in wetland rice.

There was a positive correlation between mycorrhizal colonization and grain yield. The different VAM fungi varied in their effects on plant growth and grain yield. Further, the present study confirmed previous findings that a particular host varies in its response to different VAM fungi (Vinayak and Bagyaraj 1990).

We conclude from the present study that the local fungus *G. intraradices* Po, isolated from a wetland rice soil, is the best inoculant for wetland rice among those investigated, the next best being *Acaulospora* sp. The performance of these fungi is now being tested under field conditions.

References

Dash RN, Mohanty SK, Patnaik S (1987) Measures for increasing the Indian phosphate rock for growing rice. CRRI Cuttack Annu Rep

Gangopadhyay S, Das KM (1984) Interaction between vesicular-arbuscular mycorrhiza and rice roots. Indian Phytopathol 35:34–38

Gerdemann JW, Nicolson TH (1963) Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decantation. Trans Br Mycol Soc 46:235–244

Iqbal SH, Tauquir S, Ahmad JS (1978) The effect of vesicular-arbuscular mycorrhizal associations on growth of rice (*Oryza sativa*) under field conditions. Biol Pakistan 24:357–365

Isaac SH, Kerber JD (1971) Atomic absorption and flame photometry: techniques and uses in soils, plants and water analysis. In: Walsh LM (ed) Instrumental methods for analysis of soil and plant tissue. Soil Sci Soc Am, Madison, Wisconsin, pp 17–37

Jackson ML (1973) Soil chemical analysis. Prentice Hall (India), New Delhi

Jeffries P (1987) Use of mycorrhizae in agriculture. Crit Rev Biotechnol 5:319–357

Little TM, Hill FJ (1978) Agricultural experimentation design and analysis. John Wiley and Sons, New York

Lu S, Miller MH (1989) The role of VA mycorrhiza in the absorption of P and Zn by maize in field and growth chamber experiments. Can J Soil Sci 69:97–109

Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungus for rapid assessment of infection. Trans Br Mycol Soc 55:158–161

Piper CS (1966) Soil and plant analysis. Hans Publishers, Bombay

Porter WM (1979) The most probable number for enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. Aust J Soil Res 17:515–519

Rao YSG, Bagyaraj DJ, Rai PV (1983) Selection of an efficient VA mycorrhizal fungus for finger millet: I. Glass house screening. Zentralbl Mikrobiol 138:409–413

Reena J, Bagyaraj DJ (1990) Growth stimulation of *Tamarindus indica* to selected VA mycorrhizal fungi. World J Microbiol Biotechnol 6:59–63

Secilia J, Bagyaraj DJ (1992) Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice – a preliminary screening. Mycorrhiza (in press)

Sharma AK, Singh R, Singh VA (1988) Effect of vesicular-arbuscular mycorrhiza on uptake of phosphorous and zinc in rice (*Oryza sativa* L.). Curr Sci 57:901–902

Vinayak K, Bagyaraj DJ (1990) Selection of efficient mycorrhizal fungi for trifoliate orange. Biol Agric Hort 6:305–311