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Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice – a preliminary screen

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Abstract. Eighteen different VA mycorrhizal fungi were screened for their symbiotic responses with wetland rice cultivar Prakash (IET 2254) under pot culture. Of the 18 fungi, *Acaulospora* sp. (ICRISAT), *Glomus fasciculatum* (Riverside) and *G. mosseae* (Invermay) were found to be the best in improving plant height, tiller number, total biomass, panicle number, grain weight and plant P and Zn concentrations. Increases in grain yield caused by inoculation with *Acaulospora* sp. (ICRISAT), *G. fasciculatum* (Riverside) and *G. mosseae* (Invermay) were 62%, 59% and 35%, respectively.

Key words: Rice – VA mycorrhiza – Wetland rice

Introduction

Although the beneficial effects of inoculating crop plants with vesicular-arbuscular (VA) mycorrhizal fungi are well known (Jeffries 1987; Hall 1988), rice, the most important of all the food crops in the tropics, has received little attention in mycorrhiza research. This is probably because plants growing in marshy environments, like the semi-aquatic rice, were previously considered to be nonmycorrhizal (Mmbaga 1975). Later studies showed the occurrence of VA mycorrhizal fungi in many aquatic plants, including those which are completely submerged (Chaubal et al. 1982; Clayton and Bagyaraj 1984). However, work on mycorrhizal associations of wetland rice is still very scanty (Sharma et al. 1988).

Though not host specific, recent studies have indicated host preferences of mycorrhizal fungi, thus suggesting the need for selecting efficient VA mycorrhizal fungi for a particular host (Abbott and Robson 1982; Bagyaraj et al. 1989). So far, no such study has been conducted with rice. Hence in the present investigation an attempt was made to screen and select efficient VA mycorrhizal fungi for inoculating wetland rice.

Material and methods

Seeds of wetland rice (*Oryza sativa* L.) cv. Prakash were soaked in water for 24 h and then allowed to germinate in warm, moist condition. These pregerminated seeds were used for sowing in the nursery. The soil used for raising nursery and pot culture trials was sandy-clay loam in texture with a pH of 6.9. The soil had 11.6 ppm available P (extracted with NH_4F+HCl) and an indigenous mycorrhizal population of 0.14×10^2 infective propagules/g soil.

The nursery was prepared in 20 boxes (48 cm long × 25 cm broad × 21 cm deep) of 8 kg capacity. The soil as well puddled 2 days before sowing seeds. Recommended levels of nitrogen and potassium in the form of ammonium sulphate and muriate of potash (1 kg N and 0.23 kg K/100 m^{-2}) were applied to all the pots. P in the form of superphosphate was added to 19 pots at 50% of the recommended level (0.09 kg P/100 m^2) and to one control pot at the recommended level (0.17 kg P/100 m^2).

VA mycorrhizal fungi were added to 18 pots and there were two nontreated controls, one with half the recommended P and the other with full P. Eighteen different VA mycorrhizal fungi (Table 1) obtained from various sources and maintained on *Panicum maximum* Jacq. as pot cultures were used as mycorrhizal inocula. The inocula consisting of root pieces and the sand:soil mixture were spread on the nursery beds at the rate of 50000 infective propagules/box based on the most probable number estimation (Porter 1979).

Seedlings raised in the glasshouse were transplanted 28 days after sowing. The soil used for raising nursery plants was filled into 100 plastic pots of 9 kg capacity. Healthy seedlings of uniform size were transplanted from each treatment to five pots. Two hills (planting points) at three seedlings per hill were maintained in the pots.

The plants were depotted 145 days after sowing when grain filling was complete. Plant height, tiller and panicle number were recorded at harvest. Shoot, root and grain weights were recorded at harvest. The P contents of shoot and root were estimated by the vanadomolybdate phosphoric yellow colour method (Jackson 1973). The Zn content of the plant samples was analysed after wet oxidation using an atomic absorption spectrophotometer (Hitachi model 508). The percentage mycorrhizal colonization was determined after staining the roots with trypan blue (Phillips

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Table 1. Effects of different VA mycorrhizal fungi on plant height, tiller and panicle number of rice. Values without common letters differ significantly. Significance tested by Duncan's multiple range test at $P=0.05$. IC, ICRISAT, Hyderabad, India; In, Invermay Research Station, New Zealand; Lo, Local, Hebbal, Bangalore; Nd, University of Western Australia, Nedlands; Ri, Riverside, USA; Ro, Rothamsted Research Station, England; Ru, Ruakura Research Station, New Zealand

Treatment	Plant height (cm)	Tiller number/plant	Panicle number/plant
Uninoculated (P½)[a]	30.3 cde	5.0 g	4.5 c
Uninoculated (P1)[b]	31.1 bcde	5.8 fg	4.6 c
Acaulospora laevis (In)	29.6 de	6.2 def	5.4 abc
Acaulospora sp. (IC)	35.8 a	7.5 abc	5.8 abc
Glomus albidum (Lo)	28.6 e	6.7 abcdef	5.3 abc
Glomus caledonicum (Nd)	28.9 e	7.0 abcd	6.1 ab
Glomus fasciculatum (Nd)	28.4 e	6.2 def	4.9 bc
Glomus fasciculatum (Ri)	30.7 cde	6.8 efg	4.9 bc
Glomus fasciculatum (Ro)	31.9 abcde	7.5 abc	5.7 abc
Glomus macrocarpum (Ru)	28.6 e	5.8 efg	4.5 c
Glomus merredum (Nd)	31.0 cde	6.4 cdef	5.4 abc
Glomus monosporum (Nd)	30.0 de	7.4 abc	6.2 ab
Glomus mosseae (IC)	29.4 de	5.7 fg	4.5 c
Glomus mosseae (In)	28.9 e	6.0 defg	5.3 abc
Glomus velum (Nd)	35.4 ab	6.6 bcdef	5.5 abc
Gigaspora margarita (Ru)	29.6 de	6.4 bcdef	5.3 abc
Gigaspora margarita (IC)	33.5 abcd	6.8 abcde	5.4 abc
Scutellospora calospora (Nd)	29.7 de	7.6 ab	6.4 a
Scutellospora calospora (IC)	34.3 abc	7.8 a	6.2 ab
Sclerocystis dussii (In)	29.6 de	6.5 bcdef	5.5 abc

[a] 25 kg P/ha
[b] 50 kg P/ha

and Hayman 1970). Mycorrhizal spore counts in soil were estimated by the wet sieving technique (Gerdemann and Nicolson 1963). The data was subject to statistical analysis suitable to a randomized complete block design. The treatment means were separated by the Duncan's multiple range test (Little and Hills 1978).

Results

Wetland rice cultivar Prakash varied in its response to inoculation with different VA mycorrhizal fungi. On the day of harvest, only plants inoculated with *Acaulospora* sp. showed a significant increase in plant height over the uninoculated control. The number of

Table 2. Effects of different VA mycorrhizal fungi on shoot, root, grain and total biomass of rice. Values without common letters differ significantly according to Duncan's multiple range test at $P=0.05$. Abbreviations in parentheses as in Table 1

Treatment	Dry weight (g/plant)			
	Shoot	Root	Grain	Total
Uninoculated (P½)	6.2 bcd	2.4 ef	6.0 de	14.5 g
Uninoculated (P1)	6.8 abc	2.5 ef	6.4 cde	15.7 defg
Acaulospora laevis (In)	6.0 cd	2.4 ef	7.5 bc	17.2 bcde
Acaulospora sp. (IC)	7.4 ab	4.3 a	9.7 a	20.4 a
Glomus albidum (Lo)	5.9 cd	2.6 cf	6.6 bcde	15.0 fg
Glomus caledonicum (Nd)	6.6 abcd	2.4 f	7.1 bcde	16.3 cdefg
Glomus fasciculatum (Nd)	6.3 bcd	3.3 abcd	6.3 cde	16.8 cdefg
Glomus fasciculatum (Ri)	7.1 abc	3.2 abcde	9.5 a	19.9 a
Glomus fasciculatum (Ro)	5.4 d	3.1 abcdef	6.8 bcd	15.3 efg
Glomus macrocarpum (Ru)	6.4 abcd	2.8 abcdef	5.9 a	14.9 fg
Glomus merredum (Nd)	7.3 ab	3.7 ab	7.6 bc	17.3 bcd
Glomus monosporum (Nd)	6.6 abcd	2.4 ef	7.3 bcd	16.2 defg
Glomus mosseae (IC)	6.8 ab	3.4 abcd	8.2 b	18.1 bc
Glomus mosseae (In)	7.7 a	3.1 abcde	8.1 b	18.9 ab
Glomus velum (Nd)	6.3 bcd	2.6 cdef	7.3 bcd	16.3 cdefg
Gigaspora margarita (Ru)	6.2 abc	3.0 bcdef	6.0 de	15.0 fg
Gigaspora margarita (IC)	7.1 abc	3.2 abcde	7.4 bc	17.2 bcdef
Scutellospora calospora (Nd)	6.3 bcd	3.4 abc	7.6 bc	16.9 cdef
Scutellospora calospora (IC)	6.7 abcd	2.8 bcdef	7.3 bcd	16.9 cdef
Sclerocystis dussii (In)	6.3 abc	2.4 ef	7.9 b	17.0 cde

Table 3. Effects of different VA mycorrhizal fungi on the shoot and root, P and Zn content of rice. Values without common letters differ significantly according to Duncan's multiple range test at $P=0.05$. Abbreviations in parentheses as in Table 1

Treatment	P content (mg/plant)		Zn content (μg/plant)	
	Shoot	Root	Shoot	Root
Uninoculated (P½)	12.5 f	7.3 ef	109.8 d	157.0 bcd
Uninoculated (P1)	18.8 ef	7.5 def	149.4 bcd	131.6 d
Acaulospora laevis (In)	19.4 def	11.6 abcd	202.4 ab	176.0 abcd
Acaulospora sp. (IC)	23.2 abcde	11.7 abc	175.0 abcd	204.6 abc
Glomus albidum (Lo)	21.4 bcde	9.2 cde	149.8 bcd	162.6 bcd
Glomus caledonicum (Nd)	19.2 def	8.7 cdef	151.4 bcd	158.8 bcd
Glomus fasciculatum (Nd)	19.2 def	13.6 ab	157.2 bcd	154.4 cd
Glomus fasciculatum (Ri)	28.3 ab	9.9 bcde	190.2 abc	160.4 bcd
Glomus fasciculatum (Ro)	18.6 ef	10.7 bcde	131.2 cd	139.2 d
Glomus macrocarpum (Ru)	21.2 cde	10.7 bcde	149.2 bcd	169.8 abcd
Glomus merredum (Nd)	16.8 ef	5.8 f	154.4 bcd	219.6 a
Glomus monosporum (Nd)	19.4 def	6.6 ef	155.8 bcd	177.0 abcd
Glomus mosseae (IC)	22.4 bcde	14.8 a	206.6 ab	172.4 abcd
Glomus mosseae (In)	23.8 bcde	7.6 def	172.8 abcd	201.6 abc
Glomus velum (Nd)	20.3 de	8.1 def	141.0 bcd	166.2 abcd
Gigaspora margarita (Ru)	26.3 abcde	10.1 bcde	137.2 bcd	156.4 bcd
Gigaspora margarita (IC)	18.4 ef	10.1 bcde	171.4 abcd	176.6 abcd
Scutellospora calospora (Nd)	21.2 cde	12.4 ab	163.4 abcd	196.8 abcd
Scutellospora calospora (IC)	28.2 abc	10.5 bcde	198.4 abc	174.2 abcd
Sclerocystis dussii (In)	29.9 a	7.9 def	228.2 a	212.2 ab

tillers produced was significantly higher in plants inoculated with 13 out of the 18 fungi tested. The increases in the number of panicles due to VA mycorrhizal inoculation ranged from 8.8 to 35.6% compared to the uninoculated control (Table 1), but only four values were significantly higher than the control.

The highest shoot biomass was recorded in plants inoculated with *G. mosseae* (Nd), representing an increase of 25% over uninoculated controls (Table 2). Plants inoculated with *Acaulospora* sp. showed the maximum increase in root biomass followed by *G. merredum* and *Scutellospora calospora* (Nd). Grain weight was highest in plants treated with *Acaulospora* sp. followed by *G. fasciculatum* (Ri) and *G. mosseae* (In). Plants inoculated with eight other fungi showed significant increases in grain weight ranging from 23.6 to 34.3% compared to the uninoculated control at half P. The different VA mycorrhizal fungi produced increases in total biomass (shoot + root + grain) ranging from 16 to 40%. The highest increase was in plants inoculated with *Acaulospora* sp. (40%) followed by *G. fasciculatum* Ri (37%).

Inoculation with VA mycorrhizal fungi significantly increased the P content of rice (Table 3). The shoot P content was highest in plants treated with *Sclerocystis dussii* followed by *G. fasciculatum* (Ri) and *Scutellospora calospora* (IC). Plants treated with *G. mosseae* had the highest root P content followed by *G. fasciculatum* (Nd) and *Acaulospora* sp. The shoot Zn content was highest in plants treated with *Sclerocystis dussii* followed by those inoculated with *G. mosseae* (IC), *Acaulospora laevis* and *Scutellospora calospora*. In the case of root Zn content, only plants inoculated with *G. merredum* showed a significant increase over the uninoculated control.

Table 4. Effects of different VA mycorrhizal fungi on the percentage root colonization and spore count in soil. Values without common letters differ significantly according to Duncan's multiple range test at $P=0.05$. The values for colonization are arc-sine transformed. Abbreviations in parentheses as in Table 1

Treatment	Percentage colonization	Spore count/ 25 ml soil
Uninoculated (P½)	20.9 d	76.0 h
Uninoculated (P1)	21.3 cd	42.4 h
Acaulospora laevis (In)	24.9 b	151.2 cde
Acaulospora sp. (IC)	30.1 a	252.2 a
Glomus albidum (Lo)	22.6 bcd	163.4 cde
Glomus caledonicum (Nd)	22.9 bcd	124.0 ef
Glomus fasciculatum (Nd)	21.9 bcd	128.8 ef
Glomus fasciculatum (Ri)	27.7 a	236.8 a
Glomus fasciculatum (Ro)	23.4 bcd	150.4 cde
Glomus macrocarpum (Ru)	22.3 bcd	184.6 bcd
Glomus merredum (Nd)	23.4 bcd	146.4 cde
Glomus monosporum (Nd)	22.9 bcd	132.6 ef
Glomus mosseae (IC)	24.9 b	144.2 cde
Glomus mosseae (In)	22.3 bcd	183.2 bcde
Glomus velum (Nd)	24.0 bcd	164.0 bcdef
Gigaspora margarita (Ru)	24.0 bcd	150.0 cde
Gigaspora margarita (IC)	24.9 b	140.0 ef
Scutellospora calospora (Nd)	28.6 a	102.4 fg
Scutellospora calospora (IC)	22.6 bcd	141.2 de
Sclerocystis dussii (In)	21.3 cd	192.0 b

The percentage mycorrhizal root colonization (Table 4) was highest in plants inoculated with *Acaulospora* sp. followed by *Scutellospora calospora* (Nd) and *G. fasciculatum* (Ri). The increase in spore count was highest in plants inoculated with *Acaulospora* sp. followed by *G. fasciculatum* (Ri) and *Sclerocystis dussii*.

Discussion

The important difference between a dryland and a puddled soil is the presence of oxidized and reduced soil layers in the puddled soil system (Ponnamperuma 1972). A unique characteristic of rice roots which overcomes these reduced conditions is the presence of large air spaces in mature roots. These air spaces are connected to those in the culms and leaves and provide an efficient air passage from shoot to root (Yoshida 1975). Thus the microaerophillic region around rice roots may provide a congenial environment for rhizosphere microorganisms. In the present study, colonization of rice roots grown under wetland conditions was low (14–21.6%) and agrees with the earlier findings of Manjunath et al. (1981).

Plants inoculated with VA mycorrhizal fungi were generally taller and had significantly higher tiller numbers, plant biomass, grain yield and P and Zn uptake. The species and strains of VA mycorrhizal fungi used in the present study differed in the extent to which they increased growth and P uptake of rice. The mycorrhizal parameters, viz percentage root colonization and spore count, were high in case of plants inoculated with *Acaulospora* sp. (IC) and *G. fasciculatum* (Ri), and this correlated positively with total biomass and grain weight. Significant increases in plant growth may be due to higher percentage root colonization after inoculation. The inoculum potential of the soil used in the present study was only 14 infective propagules/g soil. This implies that the soil used in the study contained low numbers and/or inefficient strains of indigenous VA mycorrhizal fungi and that the inoculated mycorrhizal fungi were relatively more efficient.

Based on the total biomass and grain yield, the best three VA mycorrhizal fungi for inoculating wetland rice were *Acaulospora* sp. (IC), *G. fasciculatum* (Ri) and *G. mosseae* (In).

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