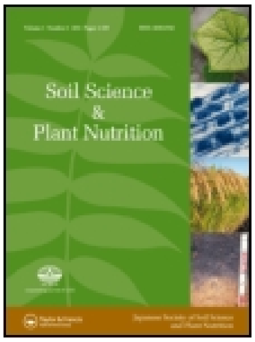


Soil Science and Plant Nutrition

ISSN: 0038-0768 (Print) 1747-0765 (Online) Journal homepage: informahealthcare.com/journals/tssp20

# Effectiveness of arbuscular mycorrhizal colonization at nursery-stage on growth and nutrition in wetland rice (*Oryza sativa* L.) after transplanting under different soil fertility and water regimes

Mohammad Zakaria Solaiman & Hiroshi Hirata

**To cite this article:** Mohammad Zakaria Solaiman & Hiroshi Hirata (1996) Effectiveness of arbuscular mycorrhizal colonization at nursery-stage on growth and nutrition in wetland rice (*Oryza sativa* L.) after transplanting under different soil fertility and water regimes, Soil Science and Plant Nutrition, 42:3, 561-571, DOI: 10.1080/00380768.1996.10416325

**To link to this article:** https://doi.org/10.1080/00380768.1996.10416325

Published online: 04 Jan 2012.

# Effectiveness of Arbuscular Mycorrhizal Colonization at Nursery-Stage on Growth and Nutrition in Wetland Rice (*Oryza sativa* L.) after Transplanting under Different Soil Fertility and Water Regimes

Mohammad Zakaria Solaiman[1] and Hiroshi Hirata[2]

*Laboratory of Plant Nutrition, Department of Biological Production, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, 183 Japan*

Received June 7, 1995; accepted in revised form October 14, 1995

**In order to analyze the effectiveness of colonization by arbuscular mycorrhizal fungi (AMF) at the nursery stage on the growth and nutrient concentration of wetland rice after transplanting, the experiments were conducted under glass-house conditions using two types of soil, namely (i) sterilized paddy soil (PS) and (ii) sterilized paddy soil diluted with sterilized Andosol subsoil 5 times (DS) under two water regimes, (i) flooded conditions changed to non-flooded conditions 30 d before harvest (F-NF) and (ii) continuous flooding (CF) up to harvest. Treatments consisting of mycorrhizal inoculation (+AMF) and non-inoculation (−AMF) were applied only at the nursery stage when the seedlings were produced under dry nursery (60% moisture of maximum water holding capacity) conditions.**

**Seedlings grown in PS showed a significantly higher biomass yield and nutrient concentrations than in DS. At 90 and 105 d after transplanting, the mycorrhizal plants showed a higher biomass than non-mycorrhizal plants in PS whereas there were no differences in DS except for roots. Mycorrhizal colonization at the transplanting stage was higher in DS than in PS. However, after transplanting opposite results were obtained, the level in PS being relatively higher than in DS. Grain yield and P concentration of unhulled grain and shoots in PS were higher in the +AMF treatments than in the −AMF treatments under both water regimes. Contents of micronutrients (Zn, Cu, Fe, and Mn) were higher in the +AMF plants than in the −AMF ones at all growth stages up to maturation irrespective of soil fertility and water regimes. These results suggest that AMF inoculation at the nursery-stage was beneficial for wetland rice after transplanting to flooded conditions in terms of growth promotion and increase of nutrient concentrations.**

*Key Words:* **arbuscular mycorrhizal fungi, growth, nursery-stage inoculation, nutrition, wetland rice.**

The beneficial effects of inoculation of crop plants with arbuscular mycorrhizal fungi

[1] Present address: Department of Soil Science, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
[2] To whom correspondence should be addressed.

(AMF) are becoming widely recognized, mainly because these fungi can improve P nutrition under certain conditions (Jeffries 1987). Recent studies have shown that plant growth and P and Zn nutrition of wetland rice were improved under pot culture conditions following inoculation with AM fungi isolated from paddy and non-paddy soils (Sharma et al. 1988; Secilia and Bagyaraj 1992, 1994). Sivaprasad et al. (1990) reported significantly higher grain and straw yields in wetland rice plants inoculated with AM fungi and cultivated by the dry nursery method. Moreover, Dhillion and Ampornpan (1992) reported that the inoculation of rice plants at the nursery stage with AM fungi was beneficial and inoculation at the nursery stage may contribute to the establishment of these plants in the field at the post-transplanting stage. In a preliminary experiment we observed that AM fungi were able to survive in wetland rice roots and soil under submerged conditions, and that the N and P concentrations of unhulled grain were increased (Solaiman and Hirata 1995). From this report, it was confirmed that for arbuscular mycorrhizal establishment, inoculation at an earlier stage or a certain interval before flooding was important. Thus an attempt was made to investigate the effectiveness of arbuscular mycorrhizal colonization at the dry nursery stage on the growth and nutrient concentrations and distribution in wetland rice plants after transplanting under flooded conditions.

## MATERIALS AND METHODS

**Sites and soils.** The soil used in this experiment included paddy soil (alluvial loamy soil, collected from Tokyo University of Agriculture and Technology farm, Fuchuhonmachi, Fuchu, Tokyo, Japan) and diluted soil. The diluted soil consisted of a mixture of paddy soil and a red Andosol subsoil in the ratio of 1 : 4 w/w. The subsoil was collected from the Tokyo University of Agriculture and Technology upland farm field, Saiwaicho, Fuchu, Tokyo, Japan. Both soils were sterilized by $\gamma$-ray irradiation (1.5 Mrad). Some of the chemical properties of the soils used in this experiment are shown in Table 1. Total N and C contents of the soils were determined with a NC analyzer (Sumitomo Chemical, SUMIGRAPH NC 80). Other soil properties were determined based on some standard methods (Page et al. 1990). Total phosphorus content in soil was determined by digestion of soil with 600 mL $L^{-1}$ perchloric acid ($HClO_4$) followed by the vanadomolybdate blue method. The available soil P was extracted by Bray II method and then the amount was measured by the vanadomolybdate blue method. The exchangeable K was extracted with 1 M neutral $NH_4OAc$ solution and the amount determined by flame photometry. Soil pH was determined in water (soil : water = 1 : 2.5 w/v) with a pH meter.

**AMF inoculum.** Fungal spores were isolated by the wet sieving and decantation method (Daniels and Skipper 1984) using a 53 $\mu$m sieve from the soil of the paddy field and inoculated at 5 cm depth from the soil surface for mycorrhizal treatment at the rate of 1,200

**Table 1.** Some chemical properties of soils used in the experiment.

| Factors | Paddy soil | Andosol subsoil | Diluted soil |
|---|---|---|---|
| pH($H_2O$) | 5.66 | 5.00 | 5.03 |
| Total carbon (g $kg^{-1}$) | 45.60 | 12.51 | 19.37 |
| Total nitrogen (g $kg^{-1}$) | 3.93 | 0.91 | 1.58 |
| Bray II P (mg $kg^{-1}$) | 187.45 | 1.83 | 38.05 |
| Total P (mg $kg^{-1}$) | 1401.60 | 51.25 | 465.20 |
| Exchangeable K (mg $kg^{-1}$) | 375.10 | 125.00 | 245.70 |

spores per box at the nursery stage. The inoculated spores consisted mainly of *Glomus* sp. The germination percentage of spores was 69.4% for 2 weeks at 25°C in steriled distilled water. Surface disinfection of spores was performed by antibiotic treatment with chloramine-T (Watrud 1984) at the rate and time exposure of 20 g $L^{-1}$ and 10 min, respectively followed by 0.25 g $L^{-1}$ streptomycin for 20 min.

**Treatment.** The treatment combinations at the nursery stage were as follows: (i) paddy soil (PS)+AMF; (ii) PS−AMF; (iii) diluted soil (DS)+AMF; and (iv) DS−AMF. The seedlings were watered daily with deionized water and kept at 60% moisture of maximum water-holding capacity during the growth period. Mycorrhizal and non-mycorrhizal seedlings of the respective treatments were transplanted to flooded pots with three replications. Plants were divided into two groups and exposed to two types of water regimes, namely (i) flooded conditions changed to non-flooded (F-NF) conditions 30 d before harvest, and (ii) continuous flooding up to harvest (CF).

**Growth of plant.** Wetland rice (*Oryza sativa* L.) var. Japonica cv. Nipponbare was cultivated for 4 weeks at the nursery stage. Surface-sterilized (by soaking in 100 g $L^{-1}$ of $H_2O_2$ for 20 min) and pregerminated seeds were sown in a nursery box at 1 week after inoculation. Eighty pregerminated seeds were sown per nursery box. The plastic nursery box (20 cm × 14 cm × 10 cm) contained 2.2 kg air dry soil. Fertilizers were applied at the rate of N 200 (urea), P 0, and K 200 (KCl) mg per nursery box as basal dressing. Four types of seedlings of the corresponding treatments were transplanted to the flooded pots of the corresponding soils with 3 replications. Two seedlings were transplanted and grown in $2 \times 10^{-4}$ a Wagner pots under glasshouse conditions from July to September 1993. The plants were watered daily with deionized water to maintain a flooding level of 3 cm from the soil surface of the pot. Fertilizers were applied at the rate of N 700 mg (urea, 300 mg as basal dressing + 200 mg at 30 d + 200 mg at 60 d after transplanting as top dressing), P 0 mg, and K 500 mg (KCl) per pot as basal dressing.

**Sampling.** Sampling was performed at 4 growth stages namely (i) early tillering stage (30 d after transplanting, DAT), (ii) maximum tillering stage (60 DAT), (iii) grain filling stage (90 DAT), and (iv) harvest stage (105 DAT). The data were collected for root colonization, plant growth parameters, and nutrient concentrations. Collected roots from the pots were washed with water and the root dry weight was recorded and a part of the fresh root sample was preserved in formalin-acetic-alcohol (FAA) for the determination of mycorrhizal colonization. The dry weight per plant of shoots (leaves and stems combined) at all sampling times was recorded. Grain yield was recorded on a 14% moisture basis including husks.

**Plant tissue analysis.** Grain (unhulled) and shoots (leaves and stems combined) were digested with concentrated (18 M) sulfuric acid ($H_2SO_4$) along with 300 mg $L^{-1}$ hydrogen peroxide ($H_2O_2$) and the contents of N, P, and K and micronutrients Zn, Cu, Fe, and Mn were analyzed. The plant P concentration was determined by the vanadomolybdate blue method, nitrogen content by the indophenol colorimetric method, and potassium by flame photometry (Page et al. 1990). Micronutrients were analyzed with an atomic absorption spectrophotometer (AAS) in the presence of Lanthanum to avoid other ionic interferences.

**Mycorrhizal assays.** The root samples were cleared in a KOH (100 g $L^{-1}$) solution at 90°C on a hot plate for 45 min to 1 h depending on the age of roots and stained with trypan blue (0.5 g $L^{-1}$) in lactoglycerol (Kormanik and McGraw 1984) at 90°C for 30 min. Percentage of colonization of the host plant roots was estimated by visual observation of stained root segments mounted in lactoglycerol by the grid-line intercept method (Giovanetti and Mosse 1980).

**Statistical analysis.** All the data were the means of 3 replications and one way analysis of variance was used to determine whether the effects of the treatment on root colonization, plant growth, and nutrient concentrations were significant in wetland rice seedlings at the nursery stage and after transplanting to the pots. Duncan's multiple range test (DMRT) or least significant difference (LSD) was used to separate the treatment means in all the tests.

## RESULTS

### AMF colonization

The colonization of roots amounted to 52.3% under DS conditions and 48.3% under PS conditions at the transplanting stage (Table 2, Fig. 1). This colonization level decreased with

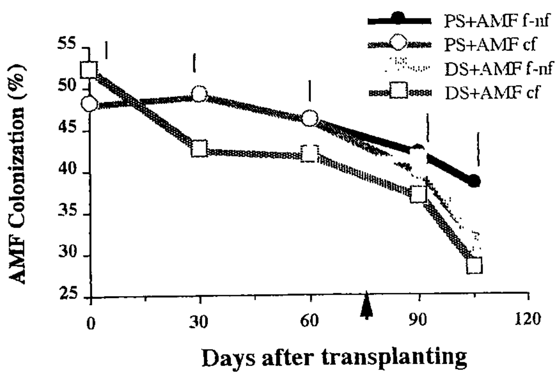


**Fig. 1.** AMF colonization of rice roots at different growth stages from nursery to maturation as influenced by soil fertility and water regimes. Non-mycorrhizal plants did not show any colonization. +AMF, arbuscular mycorrhizal fungi inoculated; PS, paddy soil; DS, diluted soil; f-nf, flooded conditions changed to non-flooded conditions 30 d before harvest; and cf, continuous flooding up to harvest. Arrow indicates the day of change of flooded (F) conditions to non-flooded (NF) conditions. Bar indicates LSD value at $p<0.05$.

**Table 2.** Effect of AMF inoculation and soil fertility on growth parameters of rice seedlings at transplanting.

| Treatment | Plant height (cm) | Shoot DW (mg plant$^{-1}$) | Root DW (mg plant$^{-1}$) | Root/shoot ratio | Total DW (mg plant$^{-1}$) | AMF colon.[a] (%) |
|---|---|---|---|---|---|---|
| PS+AMF | 41.8 a | 225.1 a | 70.7 a | 0.32 b | 295.8 a | 48.3 b |
| PS−AMF | 43.2 a | 200.0 a | 56.7 b | 0.29 b | 256.7 ab | 0 c |
| DS+AMF | 38.5 b | 148.2 b | 62.1 a | 0.42 a | 210.3 bc | 52.3 a |
| DS−AMF | 36.1 b | 131.3 b | 34.6 c | 0.26 b | 165.8 c | 0 c |

Values with similar letter(s) are not significantly different at $p<0.05$ by DMRT. [a] Colonization.

**Table 3.** Effect of AMF inoculation and soil fertility on shoot nutrient concentrations of wetland rice seedlings.

| Treatment | Macronutrient conc. (g kg$^{-1}$) | | | Micronutrient conc. (mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| | N | P | K | Zn | Cu | Fe | Mn |
| PS+AMF | 20.7 a | 8.9 a | 33.4 a | 88.5 a | 21.0 a | 365.6 a | 613.5 a |
| PS−AMF | 18.4 b | 8.8 a | 35.0 a | 59.3 c | 16.3 b | 298.3 b | 516.8 c |
| DS+AMF | 17.0 bc | 6.5 b | 30.1 b | 65.8 b | 24.6 a | 371.2 a | 565.2 b |
| DS−AMF | 15.2 c | 5.6 c | 30.5 b | 55.5 c | 23.1 a | 309.2 b | 507.9 c |

See the footnote to Table 2.

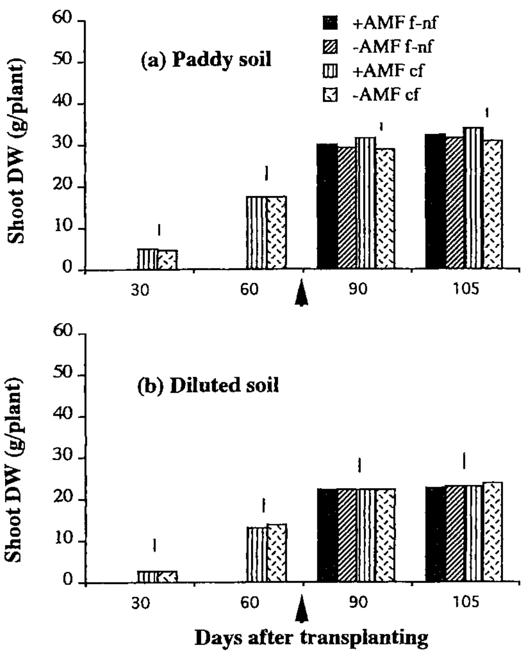


Fig. 2. Effect of AMF colonization on shoot DW (leaves+stems+grain) at different growth stages after transplanting as influenced by soil fertility and water regimes. +AMF, arbuscular mycorrhizal fungi inoculated; −AMF, not inoculated; f-nf, flooded conditions changed to non-flooded conditions 30 d before harvest; and cf, continuous flooding up to harvest. Arrow indicates the day of change of flooded (F) conditions to non-flooded (NF) conditions. Bar indicates LSD value at $p<0.05$.

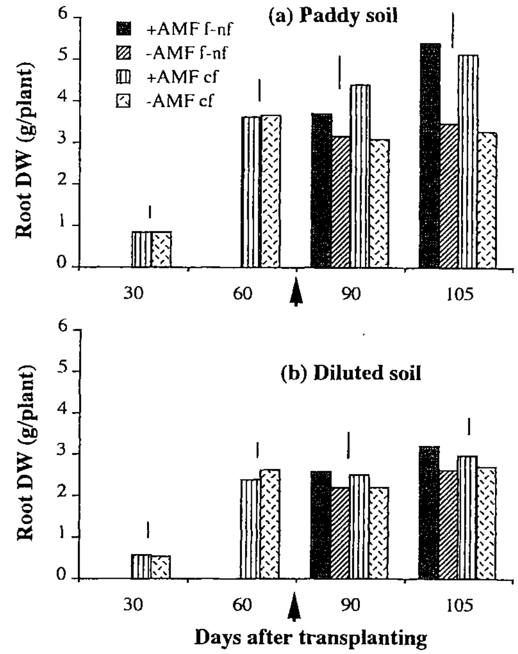


Fig. 3. Effect of AMF colonization on root DW at different growth stages after transplanting as influenced by soil fertility and water regimes. See footnote to Fig. 2.

Table 4. Effect of soil fertility and AMF inoculation on grain yield and total DW at maturation stage.

| Treatment | Unhulled grain (g plant$^{-1}$) | Total DW (g plant$^{-1}$) |
|---|---|---|
| F-NF | | |
| PS+AMF | 13.9 b | 37.9 a |
| PS−AMF | 13.4 b | 35.1 b |
| DS+AMF | 10.1 d | 25.8 c |
| DS−AMF | 9.6 d | 25.8 c |
| CF | | |
| PS+AMF | 14.8 a | 38.2 a |
| PS−AMF | 12.6 c | 34.1 b |
| DS+AMF | 9.8 d | 26.2 c |
| DS−AMF | 9.7 d | 26.7 c |

In a column, the figures having common letter(s) did not differ significantly at $p<0.05$. PS, paddy soil; DS, diluted soil; AMF, arbuscular mycorrhizal fungi; F-NF, flooded (F) conditions were changed to non-flooded (NF) ones.

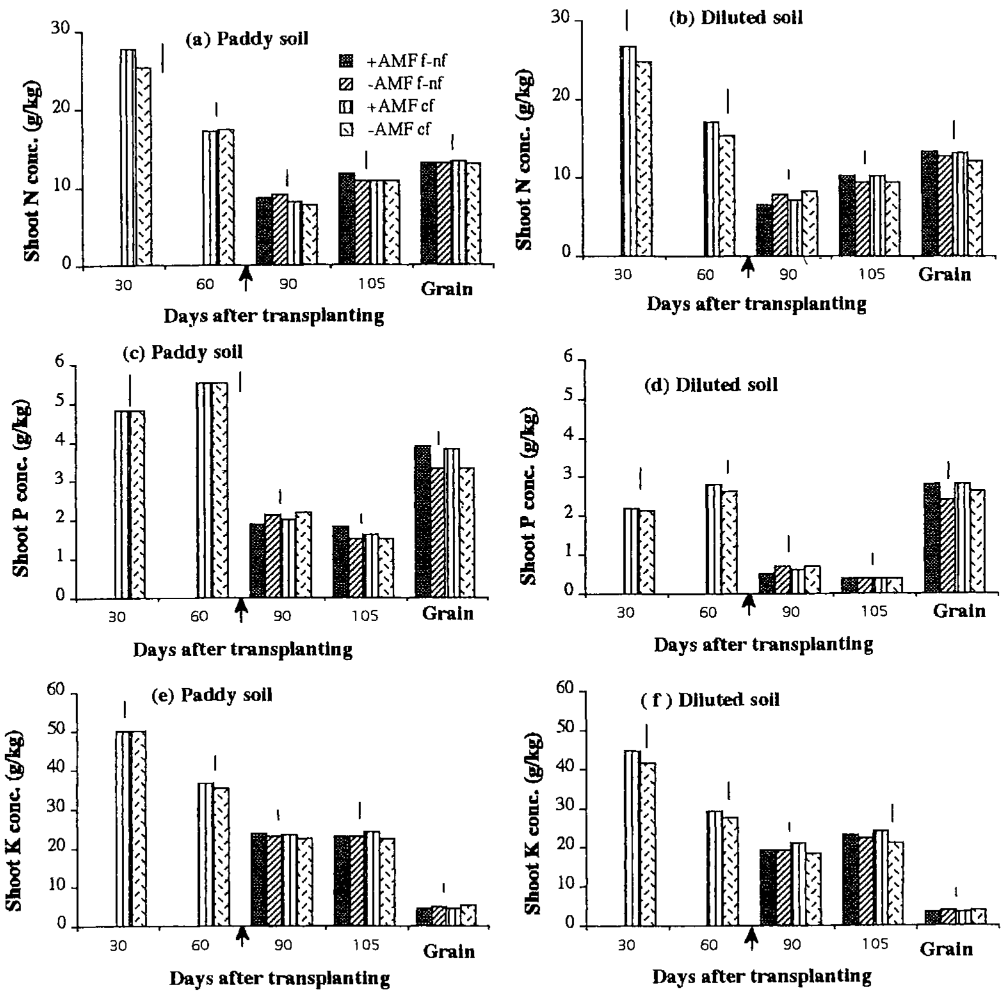


Fig. 4. Effect of AMF colonization on NPK concentrations in shoot and grain (unhulled) at different growth stages after transplanting as influenced by soil fertility and water regimes. See footnote to Fig. 2.

time along with the growth stages after transplanting and about 40% persisted in the treatment F-NF under PS conditions and about 32% under DS conditions at the maturation stage (Fig. 1). On the other hand, the colonization persisting in the CF treatment was about 32% under PS conditions whereas about 28% in DS. The colonization was significantly higher under F-NF conditions as compared with continuously flooded conditions (CF) in PS at the maturation stage. The plants not inoculated with AMF were not colonized under both soil fertility conditions and water regimes. Though the colonization level at transplanting was in the order of DS>PS, after transplanting it decreased significantly in DS, in the order of PS>DS throughout the growing stages.

## Growth and nutrition of nursery seedlings

**Growth of seedlings.** Generally DS conditions resulted in the reduction of the growth of the seedlings, although the plant height, shoot, and total DW of the seedlings were not influenced by AMF inoculation at 4 weeks after sowing (Table 2). The beneficial effect

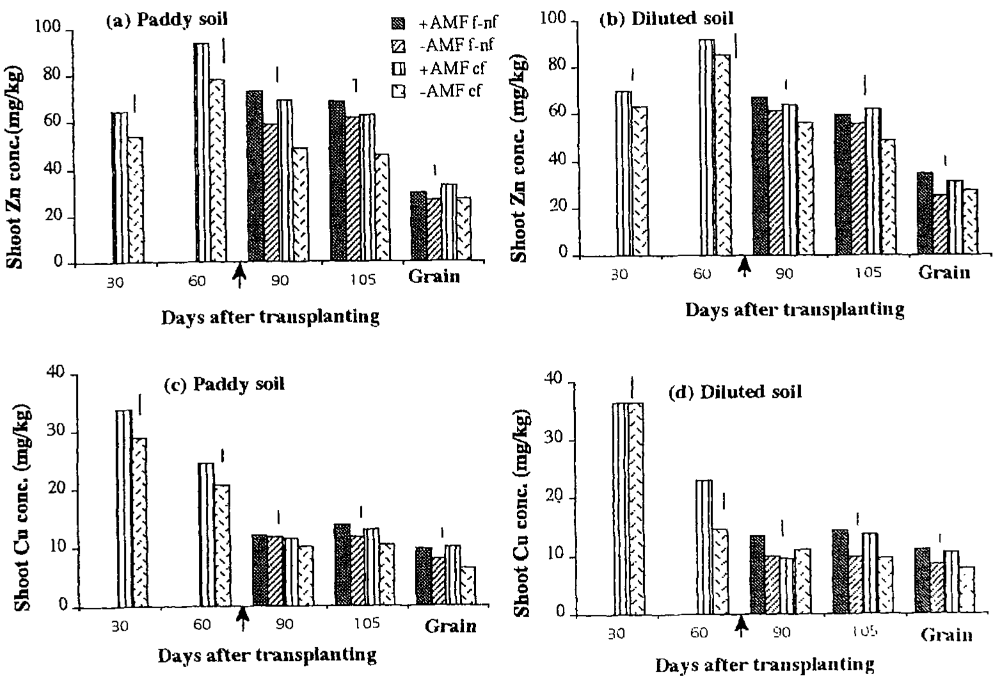


Fig. 5. Effect of AMF colonization on Zn and Cu concentrations in shoot and grain (unhulled) at different growth stages after transplanting as influenced by soil fertility and water regimes. See footnote to Fig. 2.

of AMF was observed only on root dry matter production under both soil conditions at 4 weeks after sowing. Although mycorrhizal seedlings tended to show higher root to shoot ratios than the non-mycorrhizal plants, this condition was only significant in diluted soil (Table 2).

**Shoot nutrient concentrations.** Shoot nutrient (N, P, K, Zn, Cu, Fe, and Mn) concentrations were significantly influenced by the treatment combinations used in this experiment (Table 3). The concentrations of macronutrients (N, P, and K) were all higher in PS than in DS. The shoot N, P, and micronutrient concentrations were higher in mycorrhizal plants than in non-mycorrhizal ones irrespective of soil conditions except for N and Cu in DS and P in PS at 4 weeks after sowing (Table 3). The highest Zn and Mn concentrations were obtained in the PS+AMF treatment which significantly differed from the PS−AMF treatment.

## Growth and nutrition after transplanting

**Changes in dry matter production.** In the AMF-inoculated seedlings biomass of shoots and roots was higher than in the non-inoculated ones after transplanting in PS, resulting in the highest grain yield under CF conditions at 105 d after transplanting (Figs. 2, 3, Table 4). However, the positive effect of AMF inoculated seedlings on growth after transplanting in DS was only observed on roots at the harvest stage (Fig. 3). The dry matter production in all the plant parts was significantly higher in paddy soil than in diluted soil.

**Changes in nutrient concentrations.** It was observed that shoot P concentration in DS was about 1/2 to 1/4 of that in PS throughout the growing stages irrespective of AMF

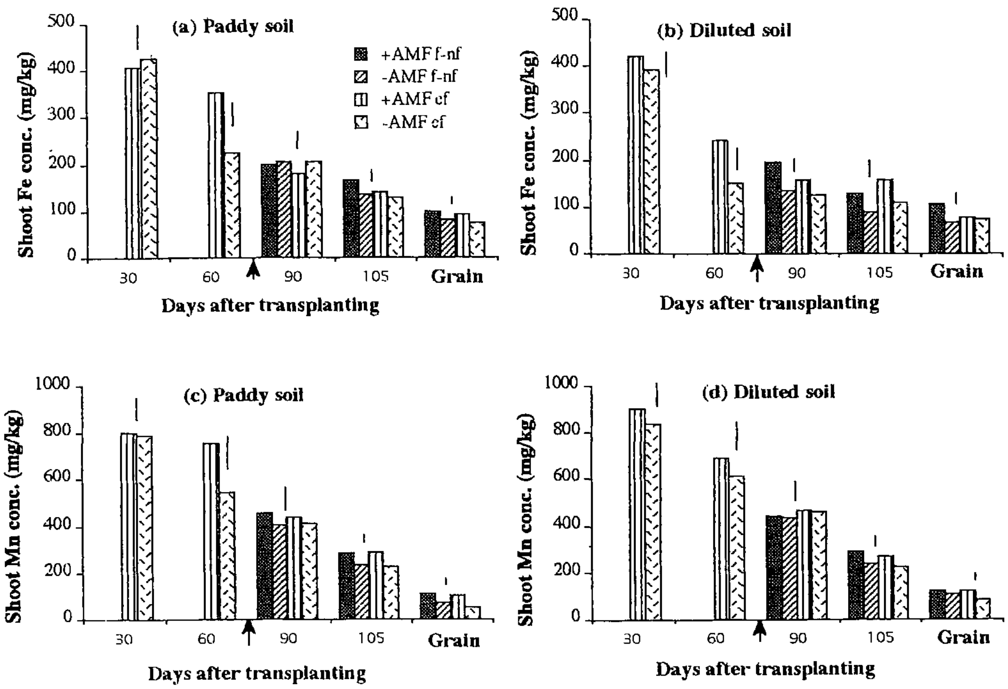


**Fig. 6.** Effect of AMF colonization on Fe and Mn concentrations in shoot and grain (unhulled) at different growth stages after transplanting as influenced by soil fertility and water regimes. See footnote to Fig. 2.

**Table 5.** Effect of soil fertility and AMF inoculation on grain and mineral nutrient harvest index at 105 d after transplanting under two different water regimes.

| Treatment | Harvest index (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Grain | N | P | K | Zn | Cu | Fe | Mn |
| F-NF | | | | | | | | |
| PS+AMF | 46.6 | 49.1 | 65.3 | 15.2 | 27.4 | 38.1 | 34.6 | 25.1 |
| PS−AMF | 46.2 | 51.2 | 66.1 | 15.3 | 26.8 | 37.3 | 33.3 | 20.9 |
| DS+AMF | 48.1 | 54.9 | 87.2 | 12.1 | 35.7 | 43.3 | 43.9 | 28.1 |
| DS−AMF | 45.3 | 53.1 | 84.2 | 12.7 | 26.6 | 41.9 | 39.7 | 24.9 |
| CF | | | | | | | | |
| PS+AMF | 48.4 | 53.3 | 68.4 | 15.6 | 33.2 | 41.8 | 38.1 | 25.1 |
| PS−AMF | 44.8 | 49.5 | 63.7 | 15.7 | 32.1 | 33.7 | 32.1 | 15.9 |
| DS+AMF | 46.4 | 52.6 | 85.3 | 10.5 | 29.7 | 39.9 | 29.4 | 28.4 |
| DS−AMF | 44.1 | 50.6 | 83.5 | 12.2 | 31.3 | 38.7 | 35.3 | 16.8 |
| LSD (5%) | 2.3 | 4.1 | 3.6 | 2.4 | 4.6 | 7.8 | 13.9 | 8.8 |

colonization. Beneficial effect of AMF inoculation on the increase in P concentration of rice was only observed in PS at the final maturation stage under both water regimes (Fig. 4c, d). Shoot and grain N and K concentrations of plants were not significantly increased by AMF inoculation at all the growth stages up to the maturation stage (Fig. 4a, b, e, f).

In general, the concentrations of micronutrients (Zn, Cu, Fe, and Mn) of the shoot were always higher with AMF inoculation throughout the growing stages irrespective of soil conditions and water regimes (Figs. 5 and 6). Zn and Cu concentrations of unhulled grain sharply increased in the AMF inoculated plants.

**Grain and nutrient harvest index.** Unhulled grain yield and harvest index (HI (%) = (grain portion)/(grain portion + straw portion) × 100) significantly increased by the AMF inoculation in DS under both water regimes whereas in PS a significant increase was recorded only under CF water regime conditions (Table 5). The N harvest index was not influenced by these treatments whereas the P harvest index significantly increased in DS and in the AMF-inoculated plants it increased in PS under CF conditions. On the other hand, the K harvest index was higher in PS than in DS. Positive effect of AMF on micronutrient distribution in grain was evident for Zn, Cu, and Mn under different medium conditions.

## DISCUSSION

The AM fungi can adapt to both low and high levels of soil nutrients. Lambert et al. (1980) who compared the performance of several AM fungi in soils with low levels of extractable P, found that plant yield was always highest when the inoculum used was indigenous to the soil in which the plants were grown. Similarly, Henkel et al. (1989) reported that adaptation to low-P soils occurred within the indigenous AMF population. On the other hand, arbuscular mycorrhizae are often present in soils with high levels of extractable P and these fungi have been considered to be P tolerant (Porter et al. 1978; Sylvia and Schenck 1983; Douds and Schenck 1990). In this experiment we used two types of soils with high (paddy soil) and low (diluted soil) fertility conditions. It was observed that both soils were suitable for mycorrhizal colonization from the nursery stage to the maturation stage under flooded conditions. AM colonization also promoted the growth of wetland rice under flooded conditions. The AM colonization level was higher in DS than in PS at the transplanting stage. However, after transplanting it became lower in DS than in PS, indicating that the development of indigenous AMF of paddy soil might be repressed in DS under submerged conditions even under low P conditions. These colonization levels were maintained just before the final maturation stage.

In the previous experiment, it was observed that inoculation performed under non-flooded conditions and maintained for a period (30 d) before flooding was important for AM establishment. The AMF inoculation increased the P and N concentrations of unhulled grain even though the AM symbiosis became parasitic to wetland rice (Solaiman and Hirata 1995). Based on the above findings, this experiment was conducted to inoculate rice under dry nursery conditions and a colonization level of about 50% was recorded irrespective of soil fertility conditions after 4 weeks of sowing. Dhillion and Ampornpan (1992) reported similar results of colonization under dry nursery conditions after a period of 45 d using rice as host plant. However, they did not report any results of post-transplanting experiment under flooded conditions.

Secilia and Bagyaraj (1992, 1994) produced mycorrhizal rice seedlings in nurseries, but no colonization level of seedlings was indicated. They also reported higher grain yield and P content in mycorrhizal wetland rice compared with non-mycorrhizal plants. Sivaprasad et al. (1990) reported significantly higher grain and straw yields in mycorrhizal wetland rice inoculated with *Glomus fasciculatum*. Gupta and Ali (1993) reported significantly higher grain yield by AMF inoculation and AMF effectiveness was equivalent to the application of half of the normal dose of phosphatic fertilizer. In our experiment it was also revealed that grain yield and the concentrations of several nutrients (P, Zn, Cu, Fe, and Mn) were higher in mycorrhizal wetland rice than in non-mycorrhizal one under flooded conditions.

After transplanting, shoot and root dry weights at earlier growth stages were not influenced by the AMF inoculation, whereas they significantly increased at 90 and 105 d in PS (Figs. 2, 3) especially root development. This fact indicated that the beneficial effect of AMF colonization at the nursery stage prevailed at the harvest stage under submerged conditions, resulting in a small increase of grain yield in CF of PS (Table 4). On the other hand, no beneficial effect of AMF on grain yield in non-flooded treatments after heading (F-NF) was observed, suggesting that the maintenance of the AMF colonization after heading may not be beneficial for grain maturation (Table 4), although it induced AMF colonized root development. The P concentrations of unhulled grain significantly increased by AMF inoculation in PS whereas in DS, the translocation of P into unhulled grain was significantly higher than in PS, which resulted in higher HI of P irrespective of treatments (Fig. 4, Table 5), reflecting the severe P deficiency in DS with Andosol subsoil. These results indicate that indigenous AM of paddy soil could not effectively promote P absorption in soils diluted with Andosol subsoil under submerged conditions as compared with dry nursery conditions.

Significantly positive effects of AMF colonization on the increase of nutrient concentration in shoots and also unhulled grain were observed throughout the growing stages of rice (Figs. 5, 6), suggesting that AMF colonization even under submerged conditions could promote the uptake of these elements as already reported (Sharma et al. 1988; Secilia and Bagyaraj 1992).

## CONCLUSIONS

Mycorrhizal inoculation at the nursery stage (60% moisture of maximum water-holding capacity) is beneficial in terms of growth and nutrition of seedlings. Nursery bed is a suitable medium for AMF establishment before transplanting of the rice plants under flooded conditions. The residual effect of AMF inoculation at the nursery stage on the growth and mineral nutrition of rice after transplanting under flooded conditions was also beneficial. It was confirmed in this experiment that higher mycorrhizal colonization level of rice roots persisted at different growth stages up to the harvest stage under continuous submerged conditions.

## REFERENCES

Daniels, B.A. and Skipper, H.D. 1984: Methods for the recovery and quantitative estimation of propagules in soil. *In* Methods and Principles of Mycorrhizal Research, Ed. N.C. Schenck, p. 29–35, The American Phytopathological Society, St. Paul, Minnesota

Dhillion, S.S. and Ampornpan, L.A. 1992: The influence of inorganic nutrient fertilization on the growth, nutrient composition and vesicular-arbuscular mycorrhizal colonization of pretransplant rice (*Oryza sativa* L.) plants. *Biol. Fertil. Soils*, **13**, 85–91

Douds, D.D. and Schenck, N.C. 1990: Relationship of colonization and sporulation by VA mycorrhizal fungi to plant nutrition and carbohydrate contents. *New Phytol.*, **116**, 621–627

Giovanetti, M. and Mosse, B. 1980: An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol.*, **84**, 489–500

Gupta, N. and Ali, S.S. 1993: VAM inoculation for wetland rice. *Mycorrhiza News*, **5**, 5–6

Henkel, T.W., Smith, W.K., and Christensen, M. 1989: Infectivity and effectivity of indigenous vesicular-arbuscular mycorrhizal fungi from contiguous soils in southwestern Wyoming, USA. *New Phytol.*, **112**, 205–214

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

Kormanik, P.P. and McGraw, A.-C. 1984: Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In* Methods and Principles of Mycorrhizal Research, Ed. N.C. Schenck, p. 29–35, The American Phytopathological Society, St. Paul, Minnesota

Lambert, D.H., Cole, H., and Baker, D.E. 1980: Adaptation of vesicular-arbuscular mycorrhizae to edaphic factors. *New Phytol.*, **85**, 513–520

Page, A.L., Miller, R.H., and Keeney, D.R. (ed.) 1990: Methods of Soil Analysis, Part 2, 2nd ed., Am. Soc. Agron., Madison, Wisconsin

Porter, W.M., Abbott, L.K., and Robson, A.D. 1978: Effect of applications of superphosphate on populations of vesicular-arbuscular endophytes. *Aust. J. Exp. Agric. Anim. Husb.*, **18**, 573–578

Secilia, J. and Bagyaraj, D.J. 1992: Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice (*Oryza sativa* L.) plants. *Biol. Fertil. Soils*, **13**, 108–111

Secilia, J. and Bagyaraj, D.J. 1994: Selection of efficient vesicular-arbuscular mycorrhizal fungi for wetland rice—A preliminary screen. *Mycorrhiza*, **4**, 265–268

Sharma, A.K., Singh, R., and Singh, V.A. 1988: Effect of vesicular-arbuscular mycorrhiza on uptake of phosphorus and zinc in rice (*Oryza sativa* L.). *Current Sci.*, **57**, 901–902

Sivaprasad, P., Sulochana, K.K., and Salam, M.A. 1990: Vesicular-arbuscular mycorrhizae (VAM) colonization in lowland rice roots and its effect on growth and yield. *Int. Rice Res. Newslett.*, **15**, 14–15

Solaiman, M.Z. and Hirata, H. 1995: Effects of indigenous arbuscular-mycorrhizal fungi in paddy fields on rice growth and N, P, K nutrition under different water regimes. *Soil Sci. Plant Nutr.*, **41**, 505–514

Sylvia, D.M. and Schenck, N.C. 1983: Application of superphosphate to mycorrhizal plants stimulates sporulation of phosphorus-tolerant vesicular-arbuscular mycorrhizal fungi. *New Phytol.*, **95**, 655–661

Watrud, L.S. 1984: Spore germination and axenic culture of endomycorrhizae. A. Spore germination of vesicular-arbuscular mycorrhizal fungi. *In* Methods and Principles of Mycorrhizal Research, Ed. N.C. Schenck, p. 81–83, The American Phytopathological Society, St. Paul, Minnesota