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Comparing the Effect of Arbuscular Mycorrhizal Fungi on Upland Rice and *Macaranga denticulata* in Soil with Different Levels of Acidity

Somchit Youpensuk[a*], Sittichai Lordkaew[b] and Benjavan Rerkasem[c]

[a] Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.
[b] Multiple Cropping Centre, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.
[c] Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

* Corresponding author, E-mail: scboi027@chiangmai.ac.th

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ABSTRACT: *Macaranga denticulata* Muell. Arg. is a fallow-enriching tree in upland rice fields where rotational shifting cultivation is practical. It was found associated with high diversity of arbuscular mycorrhizal (AM) fungi in the village of Haui Tee Cha, Sob Moei District, Mae Hong Son Province of northern Thailand. Soils in the village had low available P (about 2-4 mg/kg by Bray II), and pH ranging from about 4.5 to 6.0. This study evaluated the effects of AM fungi on upland rice in comparison with *M. denticulata* and root colonization in the host plants in three levels of soil pH, 4.5, 5.9 and 7.8, in a pot experiment. The optimal soil pH for growth of upland rice and *M. denticulata* were 4.5 and 5.9, respectively. In spite of almost no difference in the percentage root colonization and spore density, the effect of AM on growth of upland rice was very different from that on *M. denticulata*. AM fungi had no effect on dry weight and grain yield of upland rice. But in *M. denticulata*, AM fungi increased dry weight and nutrient contents, especially at pH 4.5 and 7.8. However, AM fungi significantly increased the nutrient content in seeds of upland rice in pH 7.8 soil. These results indicate that AM fungi play an important role in the maintenance of soil fertility and upland rice productivity in shifting cultivation by enhancing accumulation of nutrients in the fallow enriching *M. denticulata*.

KEYWORDS: arbuscular mycorrhizal fungi, fallow enrichment, *Macaranga denticulata*, shifting cultivation, soil acidity, upland rice.

INTRODUCTION

The highlands of northern Thailand are populated by many ethnic minority groups. The people originally made a living from slash and burn agriculture. Rotational shifting cultivators typically settled in one place to grow rice and associated crops in a system of rotation involving one year of cropping and about 10 years of fallow[1]. *Macaranga denticulata* Muell. Arg. is a fallow-enriching tree that is well known among the various ethnic groups who make a living on rotational shifting cultivation, including those who live in the village of Haui Tee Cha, Sob Moei District, Mae Hong Son Province of Northern Thailand[2]. Youpensuk *et al*.[3] found a very high diversity with 29 species of arbuscular mycorrhizal (AM) fungi in the root zone of *M. denticulata*. The diversity of AM fungi in the root zone of upland rice was found to be about half of that in the root zone of *M. denticulata* [4]. The soils in this village had low available P (about 2-4 mg/kg by Bray II), and pH ranging from about 4.5 to 6.0. On these very poor soils, Karen farmers in the village of Haui Tee Cha achieved much higher upland rice yield with dense stands of *M. denticulata* (4000 trees/ha) compared with fallow plots in which the trees had been poorly established (1000 trees/ha) [2].

Arbuscular mycorrhizal fungi are soil fungi colonizing the roots of plants belonging to over 90% of plant families[5]. They benefit host plants with increased nutrient uptake, especially P, and withstand many environmental conditions[6]. Many AM fungi are effective over a wide pH range[7]. However, differences in root colonization are frequently observed with different plant-fungus combinations[8]. Youpensuk *et al*.[3] found that AM fungi were effective for growth and nutrient uptake of *M. denticulata*. However, the effect of AM fungi on upland rice was still not known.

The experiment had two objectives: (1) to evaluate the effect of soil pH on root colonization of AM fungi in the host plants, (2) to determine the effects of soil pH and AM fungi on growth and nutrient contents of upland rice in comparison with *M. denticulata*, previously shown to be highly responsive to infection by AM fungi [3].

MATERIALS AND METHODS

Experimental Design

The experiment was a full factorial of three levels of soil pH (4.5, 5.9 and 7.8) and two treatments of AM

inoculation (inoculated and uninoculated treatments) with two species (rice and *M. denticulata*), in four replications. The soil used in this experiment was a sandy loam textured Typic Tropaqualf of the San Sai series. It had 57% sand, 24% silt and 19% clay.

Soil Description

The soil contained 0.9 g/kg total N (Kjeldahl method), 4.1 mg/kg available P (Bray II method), 53.0 mg/kg extractable K (1 *N* NH_4OAc, pH 7) and 18.7 g/kg organic matter (Walkley-Black method). It had a pH of 5.9. The soil pH was adjusted to 4.5 and 7.8 by mixing with $Al_3(SO_4)_2.18H_2O$ and $CaCO_3$ at the rates of 1.5 and 5.0 g/kg soil, respectively. All of the soil used in the experiment was steamed at 100°C for four hours twice with an interval of 24 hours.

Growing Conditions

Seeds of upland rice (local variety called *Bue Bang*) and *M. denticulata* from the village of Haui Tee Cha were surface sterilized in 1.2% sodium hypochlorite for five minutes and washed twice with sterile water. Seeds were sown in pots containing five kg of soil. After emergence, upland rice seedlings were thinned to two plants per pot and *M. denticulata* seedlings were thinned to one plant per pot. AM fungal spores were obtained from soil in the village of Haui Tee Cha, Sob Moei District, Mae Hong Son Province, Thailand by wet sieving and 50% sucrose centrifugation.[10] Spores in the supernatant were poured over a 40 mm sieve and washed with water to remove the sucrose before vacuum filtration on filter paper. Spores on filter paper were kept in Petri dishes. The AM fungal spores were selected with fine tip forceps under a stereomicroscope and about 300 spores were transferred to each sterile moist filter paper in a small plate. For AM inoculated treatments, the spores of AM fungi from one small plate were inoculated to each pot. The pots were placed in a green house at Chiang Mai University. Every pot was watered once a day with filtered tap water about 500 ml per pot.

Nutrient and Mycorrhizal Assessment

The experimental plants were harvested at four months. Dry weight was determined separately for shoots and roots, and also seed in the case of rice. Shoots, straw and seeds were analysed for N (Kjeldahl method), P (Dry ashing and molybdovanado-phosphoric acid method) and K (Dry ashing and atomic absorption spectrophotometry method) contents.

Soil samples were collected by coring four soil cores (3 cm diameter) in each pot from the soil surface to the bottom of the pot, midway between the stem and the pot wall. Two soil cores of each pot were used to determine spore density and the root sample in these soil cores was used to determine root colonization. Root samples were washed and cut into pieces 1-2 cm in length. The root samples were cleared in 10% KOH at 121°C for 5 minutes for upland rice and 15 minutes for *M. denticulata* and washed with water. Cleared roots were stained with 0.05% trypan blue in lactoglycerol at 121°C for 5 minutes for upland rice and 15 minutes for *M. denticulata*. Thirty pieces of stained roots from each sample were mount on glass slides to assess root colonization by AM fungi (McGonigle *et al.*, 1990 method).[11] Spores of AM fungi were separated from 2×25 g dry soil of each soil sample by wet sieving and 50% sucrose centrifugation.[10] Spores were kept on filter paper with gridlines in Petri dishes and counted under a stereomicroscope. Roots in the other two soil cores were washed and dried to determine root dry weight in the soil cores. The remaining roots in pots were washed and dried to measure dry weight.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) for a completely randomized design. Residuals were normally distributed with constant variance. SPSS software programs were used to conduct the ANOVA. Significances of treatment means were determined at $P \leq 0.05$ with Duncan's Multiple Range Test.

Results and Discussion

Growth and Nutrients Uptake of Upland rice

The highest upland rice growth was found at the soil pH 4.5, and the lowest growth of upland rice was found at pH 7.8 (Table 1). Upland rice grown in pH 4.5 soil had significantly greater root weight than rice grown in pH 5.9 and 7.8 soils. This was reflected in higher nutrient uptake at the lowest pH (Table 2). Root to shoot ratios were also lower in the higher soil pH. These results may be due to selection of this Bue Bang upland rice variety by Karen farmers for adaptation to acidic soil in the village of Haui Tee Cha. Growth of upland rice was not significantly different between AM inoculated and uninoculated treatments. In this study, the upland rice variety Bue Bang from the village of Haui Tee Cha did not respond significantly to the indigenous AM fungi although it had high AM colonization (Fig 1). The benefits resulting from the inoculation of plants with mycorrhizal fungi may be high or low depending on the properties of the host plant, AM fungi or soil where they are grown [10]. Plants with highly branched, fine, long roots with numerous root hairs have often been observed to derive low benefit from mycorrhizas [12, 13]. The root system of upland rice is composed of fibrous roots that are fine, long, highly branched, and abundant with root hairs. Therefore, they efficiently uptake

Table 1. Effects of soil pH and AM fungal inoculation on seed number, shoot and root dry weight (DW) of upland rice.

Soil pH	Inoculation	Seed weight (g/plant)	Straw DW (g/plant)	Root DW (g/plant)	Root: shoot ratio
4.5	M	3.17a	5.69a	4.87a	0.5:1
4.5	NM	3.54a	5.48ab	5.13a	0.6:1
5.9	M	2.98a	3.33c	2.43b	0.4:1
5.9	NM	2.66ab	3.62bc	2.80b	0.4:1
7.8	M	1.90bc	1.89c	1.59b	0.4:1
7.8	NM	1.25c	1.63c	0.90b	0.3:1
Analysis of variance					
Soil pH		***	***	***	
Inoculation		NS	NS	NS	
pH × Inoculation		NS	NS	NS	

M, inoculated; NM, non-inoculated with AM fungi.
Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test.
***, significant at $P \leq 0.001$; NS, not significant at $P \leq 0.05$.

Table 2. Effects of soil pH and AM fungal inoculation on above ground nutrient contents of upland rice.

Soil pH	Inoculation	Above ground nutrient content (mg/plant) N	P	K
4.5	M	79.36a	5.96a	111.77ab
4.5	NM	69.67ab	6.01a	121.62ab
5.9	M	60.90bc	7.39a	128.30a
5.9	NM	56.37c	6.48a	101.94ab
7.8	M	42.27d	6.92a	84.32bc
7.8	NM	27.60e	2.01b	59.90c
Analysis of variance				
Soil pH		***	**	**
Inoculation		**	***	NS
pH × Inoculation		NS	**	NS

M, inoculated; NM, non-inoculated with AM fungi.
Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test.
*** and **, significant at $P \leq 0.001$ and 0.01, respectively; NS, not significant at $P \leq 0.05$.

Table 3. Effects of soil pH and AM fungal inoculation on seed nutrient contents of upland rice.

Soil pH	Inoculation	Seed nutrient content (mg/plant) N	P	K
4.5	M	32.63a	3.98ab	10.31a
4.5	NM	33.57a	3.65b	10.45a
5.9	M	28.98b	4.69a	9.61ab
5.9	NM	26.13c	4.32ab	9.38ab
7.8	M	19.57d	4.32ab	8.36b
7.8	NM	13.10e	1.32c	3.50c
Analysis of variance				
Soil pH		***	***	***
Inoculation		***	***	***
pH × Inoculation		***	***	***

M, inoculated; NM, non-inoculated with AM fungi.
Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test.
***, significant at $P \leq 0.001$.

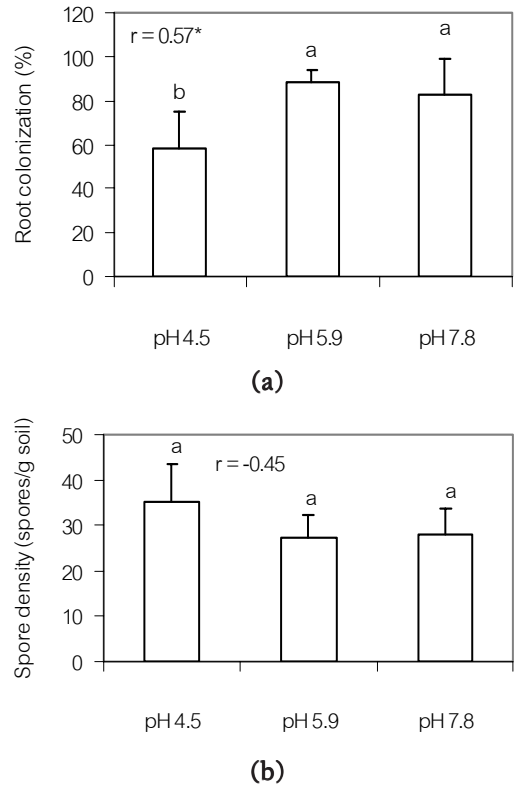

Fig 1. Means of root colonization (a) and spore density (b) in roots of upland rice at different soil pH. Columns with different letters indicate a significant difference in the means. Bars are ± standard error of the means.

nutrients without AM colonization. Dhillion and Ampornpan[14] reported significantly reduced shoot and root dry weight in AM inoculated rice compared to non-AM plants when the plants were supplied with P and N. Dhillion[15] reported that inoculation by AM fungi resulted in significant differences in proportions of root colonization and growth among various AM fungus-rice variety combinations and uninoculated plants. Soil pH had a significant effect on above ground nutrient contents, for example N contents of upland rice grown in low pH soil were higher than those in high pH soil. Inoculation with AM fungi significantly increased N and P contents of upland rice, especially at the pH 7.8 soil (Table 2). AM fungi significantly increased N content of upland rice seeds in the pH 5.9 and 7.8 soils, and significantly increased P and K in the pH 7.8 soil (Table 3). Although AM fungi did not significantly increase upland rice growth and yield, they increased nutrient contents in the rice seeds, and so may help to improve the nutritional quality of upland rice for those who grow rice for subsistence. For example, it has been suggested that improving milled rice protein by 2% (from 7 to 9%) would double the protein intake in the Asian diet from 10 to 20% [16].

Growth and Nutrients Uptake of *Macaranga denticulata*

Arbuscular mycorrhizal fungi had a much greater effect on dry weight and nutrient uptake of *M. denticulata* than they did for rice. The effect, however, varied with pH treatments. Soil pH had a significant effect ($P<0.001$) on height and shoot and root dry weight of *M. denticulata* (Table 4). In uninoculated treatments, growth of *M. denticulata* was very stunted, especially in pH 4.5 and 7.8 soils, and some plants died within three months. In the inoculated treatments, growth of the host plant in the pH 7.8 soil was significantly lower than the other treatments. The highest growth of *M. denticulata* was found in the pH 5.9 soil with AM inoculation (Table 4). This showed an interaction effect of AM inoculation and the suitable soil pH (5.9) for growth of *M. denticulata*. Arbuscular mycorrhizal fungi enhanced the ability of the host plant to grow and withstand unsuitable soil pH. *Macaranga denticulata* was highly mycorrhizal dependent, especially at the sub-optimal pH of 4.5 and 7.8. The results may be related to *M. denticulata* having a coarse root system with few root hairs. The wide dispersal of mycorrhizal hyphae in soil, and the small diameter of hyphae relative to roots, gives access to a much larger volume of soil than the root system itself [17]. Inoculation with AM fungi increased plant growth and the N, P and K concentrations in the host plant (Table 5). The highest N, P and K concentrations in host plants were in those growing in the pH 5.9 soil, and soil with the highest root colonization of AM fungi (Fig 2). In very low pH soil or alkali soil, plants normally encounter toxicity and deficiency of some nutrient elements [18]. In the inoculated treatments, the AM fungi appeared to protect the host plant from toxicity and enhance the host plant's acquisition of nutrients. Extremely acid soils (pH 4.0-5.0) can have high concentrations of soluble Al, Fe, Zn, Cu and Mn, which may be toxic to the growth of some plants [19]. Zhu *et al.* [20] compared Zn uptake of mycorrhizal and non-mycorrhizal white clover plants grown in sterile soil with five application rates of Zn (as $ZnSO_4$) from 0 to 400 mg/kg. Increasing the Zn application rate led to increased uptake of Zn in plants, but the increases were significantly greater in non-mycorrhizal plants than in mycorrhizal plants. This suggests that metals may be sequestered in the hyphae and not transferred to the plant[8], while in alkaline soils, in which P will be less available and Zn deficiencies are occasionally observed in some sensitive plants, the uptake of minerals by the roots is supplemented by mycorrhizal fungi. Arbuscular mycorrhizal fungi increase minerals that are poorly available, immobile in soil, and depleted around the root. The fungi also influence availability by changing the movement of available organic energy from the root into the soil [21]. Hyphae of AM fungi act as a pump,

Table 4. Effects of soil pH and AM fungal inoculation on height, shoot and root dry weight (DW) of *M. denticulata*.

Soil pH	Inoculation	Height (cm)	Shoot DW (g/plant)	Root DW (g/plant)	Root: shoot ratio
4.5	M	10.63a	2.80b	2.35b	0.8:1
4.5	NM	2.50c	0.02d	0.01d	0.5:1
5.9	M	12.00a	4.09a	3.72a	0.9:1
5.9	NM	6.25b	1.21c	1.28c	1.1:1
7.8	M	7.13b	1.30c	0.83c	0.6:1
7.8	NM	1.83c	0.01d	0.01d	1.0:1
Analysis of variance					
Soil pH		***	***	***	
Inoculation		***	***	***	
pH × Inoculation		*	*	**	

M, inoculated; NM, non-inoculated with AM fungi.
Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test.
***, ** and *, significant at $P\leq0.001$, 0.01 and 0.05, respectively.

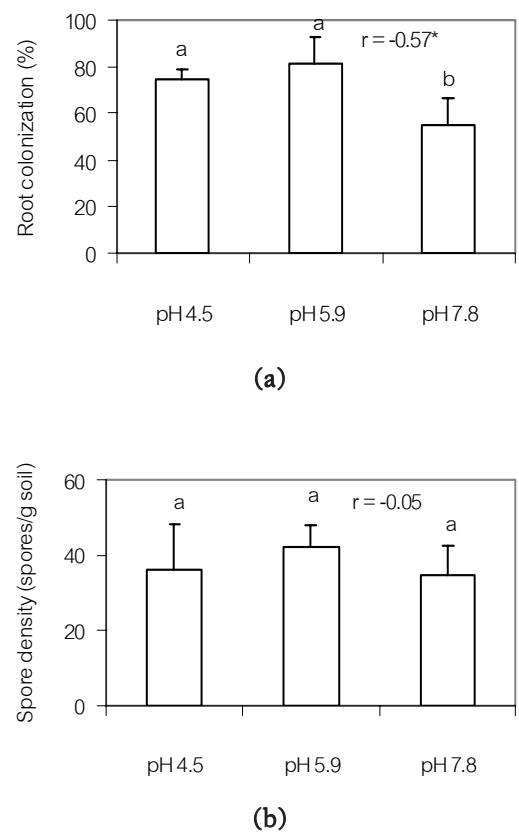

Fig 2. Means of root colonization (a) and spore density (b) in roots of *M. denticulata* at different soil pH. Columns with different letters indicate a significant difference in the means. Bars are ± standard error of the means.

Table 5. Effects of soil pH and AM fungal inoculation on shoot nutrient contents of *M. denticulata*.

Soil pH	Inoculation	Shoot nutrient content (mg/plant) N	P	K
4.5	M	56.75a	4.25b	42.25b
4.5	NM	nd	0.01d	0.10d
5.9	M	66.75a	5.75a	52.75a
5.9	NM	14.50c	2.00c	13.00c
7.8	M	27.25b	2.25c	20.25c
7.8	NM	nd	0.01d	0.07d
Analysis of variance				
Soil pH		***	***	***
Inoculation		***	***	***
pH × Inoculation		***	*	**

M, inoculated; NM, non-inoculated with AM fungi.
Means in the same column followed by different letters are significantly different by Duncan's Multiple Range Test.
***, ** and *, significant at $P \leq 0.001$, 0.01 and 0.05, respectively;
nd, not detected because there were not enough of the samples.

supplying the root with mineral salts to which it normally would not have full access [17].

Root Colonization and AM Spore Association with Upland Rice and *M. denticulata*

The percentage of root colonization in upland rice in the pH 4.5 soil of 58% was significantly lower than in the other treatments. The highest root colonization in roots of upland rice was 87% in the pH 5.9 soil. Percentages of colonization are often related to differences in rates of root growth and susceptibility of the plants, as well as to different fungal strategies in root colonization[8]. In this experiment, upland rice in the pH 4.5 soil had a higher growth rate of roots than in the pH 5.9 and 7.8 soils as evidenced by root dry weight, which was significantly higher than other treatments. Therefore, the percentage of root colonization by AM fungi in the pH 4.5 soil was significantly lower than other treatments.

Root colonization of AM fungi associated with *M. denticulata* in the pot experiment ranged from 55 to 81%. The optimum soil pH for root colonization of AM fungi in *M. denticulata* was 5.9. It was the same pH as in roots of upland rice. The lowest root colonization was found in the pH 7.8 soil. The root growth rate of *M. denticulata* is very slow compare to the root growth rate of upland rice. Therefore, environmental conditions have important influences on percentage colonization. Some AM fungi prefer acidic to alkali soils [19]. In this experiment, AM fungi association with *M. denticulata* in the pH 7.8 soil was not well establish compared to the other pH soils. Root colonization had positive effects on growth of the host plant.

Soil pH did not affect spore densities of AM fungi in both root zones of upland rice and *M. denticulata* (Figs 1 and 2). Spore densities in the root zone of upland rice in the pH 4.5, 5.9 and 7.8 soils were 35, 27 and 28 spores/g dry soil, respectively, while spore densities in the root zone of *M. denticulata* at these soil pH were 36, 42 and 35, respectively.

Inoculum used in this experiment was mixed species of AM fungi. Therefore, spore production was determined by the proliferation ability of individual species all in each soil condition. Many researchers reported that there was no relationship between spore density and root colonization[8, 22, 23].

Conclusion

The optimal soil pH for growth was 4.5 for upland rice and 5.9 *for M. denticulata*. Both upland rice and *M. denticulata* had high root colonization by AM fungi at the soil pH 5.9, but upland rice growth did not respond to root colonization. However, AM fungi significantly increased N content in seeds of upland rice in the pH 5.9 and 7.8 soils, and significantly increased P and K in the pH 7.8 soil. *Macaranga denticulata*, on the other hand, was highly mycorrhizal dependent for growth and N, P and K uptake. As *M. denticulata* is a pioneer species, known for its fallow enriching property, nutrients from plant litters are restored to the soil and benefit rice yields by nutrient cycling. Upland rice, on the other hand, appeared to benefit directly from association with the mycorrhizal fungi only marginally.

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