CHAPTER 4

THE SYMBIOSIS OF ARBUSCULAR MYCORRHIZAL FUNGI IN UPLAND AND LOWLAND RICE IN FREELY DRAINED SOIL

4.1 Introduction

The previous pot trials indicate that AM fungi may be important for growth and nutrient uptake in a range of food crops, including upland rice, grown in a shifting cultivation system. Generally, plant response to AM inoculation is particularly strong in soils of low P status (Chapter 3; Youpensuk *et al*., 2004; Youpensuk *et al*., 2006). For example, upland rice (cv. Bue Bang) responded to inoculation by increasing seed yield at low P (3.4 mg kg^{-1}, Bray II). The results in Chapters 2 and 3 relate to rain-fed farming systems and little is known about the role of AM fungi in lowland rice in Thailand.

Rice grown under waterlogged conditions has greater P uptake than when grown under dry conditions (Insalud, 2006). This may be due to P availability increasing under flooded conditions (Ponnamperuma, 1972). By contrast, P availability decreases under aerobic conditions and P deficiency is therefore a concern for upland rice production in Thailand. Many Thai farmers do not have the economic capacity to apply sufficient P fertilizer to maximize rice yields. Hence, P deficiency is often a factor limiting crop production.

It is well known that AM fungi increase growth of many plant species by enhancing nutrient uptake. The most understood benefit of AM fungi is the uptake

and transfer of nutrients N, P, K, Ca, Mg, Cu, Mn and Zn to the host (Marschner and Dell, 1994). Of these, P is of particular interest due to its soil chemistry and depletion in the rhizosphere.

Understanding the associations between AM fungi and rice may be useful for managing beneficial AM fungi in order to improve plant nutrient uptake. The resulting benefit to plant growth may help farmers to increase their seed yields and/or seed quality and decrease inorganic fertilizer inputs especially on infertile soil.

Rice production in many parts of Thailand is rain-fed, including both upland and lowland rice cropping (DOA). Finding out how rice responds to AM fungi would be useful to farmers who grow rice under rain-fed conditions. The aims of this study were to examine the role of AM fungi on growth, yield and seed nutrient concentrations of lowland rice (KDML 105) in comparison to upland rice (Bue Bang) in a freely drained soil. KDML 105 is a popular variety grown on 26% of Thailand's rice land (OAE, 1998).

4.2 Materials and Methods

Experimental design

The experiment was conducted in CRD with factorial combination of 2 rice varieties, 2 levels of P and 3 inoculation treatments with four replicate pots.

The P treatments were 1 and 10 kg P ha^{-1}, hereafter defined as P1 and P10, applied as finely ground triple superphosphate, giving 4.9 and 7.0 mg P kg^{-1}, respectively, Bray II P at 15 weeks of incubation.

The inoculum treatments were: (1) control (soil inoculum that had been autoclaved at 121 °C for 60 minutes: AM0), (2) inoculated with *Scutellospora* spp.

250 spores plant^{-1}: AM1 [this fungus was used because it was able to be produced from single spore culture (see Appendix) and it was present in the rhizosphere of rice and pada in farmers' fields] and (3) inoculated with mixed AM population: AM2 (25 g plant^{-1} of soil inoculum from pada rhizosphere as Chapter 3). The soil inoculum (AM fungi + possibly other beneficial organisms) from Huai Tee Cha village was used for this study to enable comparison of responses to previous chapters. The experiment was conducted in an outdoor screen house consisting of a plastic roof and mesh walls, from August (rainy season) to November (cool season) 2006, at the Agronomy Department, Chiang Mai University.

Seed and soil preparation

Seed of upland rice (cv. Bue Bang), taken from a single panicle, were brought from a farmer at Huai Tee Cha village and lowland rice (cv. KDML105) was obtained from the Rice Department. The seeds were surface sterilized in 70% ethanol for five minutes and washed five times with sterile water.

The soil (3.03 mg kg^{-1} Bray II P) was collected from the same field as in Chapter 3. The Sansai soil pH was 6.05 and was adjusted to 5.2 with $Al_2(SO_3)_4.18H_2O$ so it was similar to the soil medium in Chapter 3. It was mixed with coarse river sand (2:1 v/v), the finely ground triple superphosphate was mixed throughout the dry soil and then it was steam-pasteurized at 95 °C for five hours and repeated after 24 hrs. Six seeds per pot (2 seeds hole^{-1}) were planted at a depth of 2 cm and the inoculum was placed under the seed. Plants were grown in plastic pots (21 cm top diameter, 14 cm bottom diameter, and 16 cm depth) with basal drainage holes, placed on raised mesh beds. Basal nutrients were applied as in Chapter 3. The

pots were watered with filtered tapwater to field capacity daily with minimum leaching. Plants were thinned to three plants pot^{-1} 3 days after emergence.

Harvest

Crops were harvested fourteen weeks after sowing, at grain maturity. Plant height was measured until maximum height was obtained (at the ninth week). Prior to the harvest, four soil cores (3 cm diameter) were taken from the soil surface to the bottom of the pot, mid-way between the plant and the centre of the pot, and combined into one sample for spore analysis (Figure 3.1, Chapter 3). The shoot was partitioned into stems, leaves, seeds and the roots were washed free of soil. The roots were subsampled for determining root colonization and examination of spores (Chapter 2). All plant parts were dried at 75 ^{o}C for 48 hours to measure dry weight and then were analysed for N by the Kjeldahl method (Jackson, 1967), P by dry ashing followed by the molybdovanado phosphorus acid method (Murphy and Riley, 1962) and K by dry ashing and atomic absorption spectrophotometry. Brown rice and husk were analyzed for nutrient concentration (P, K, S, Ca, Cl, Na, Mg, Fe, Zn, Cu and Mn) by ICP-AES (Inductively coupled plasma-atomic emission spectroscopy) at Murdoch University in March – May, 2007. Nitrogen concentration in brown rice and husk was analyzed by the Kjeldahl method at CMUPN*lab*.

Data analysis

Data were analyzed by using commercial software (Statistix V. 8, Analytical Software, Inc.). Total dry weights, nutrient uptake, seed weight, nutrient

concentrations in brown rice or husk and spore density were analyzed by analysis of variance (ANOVA). Percentage data for root colonization were arcsine transformed. Least significant difference (LSD) at 5% confidence level was used for comparison under ANOVA.

4.3 Results

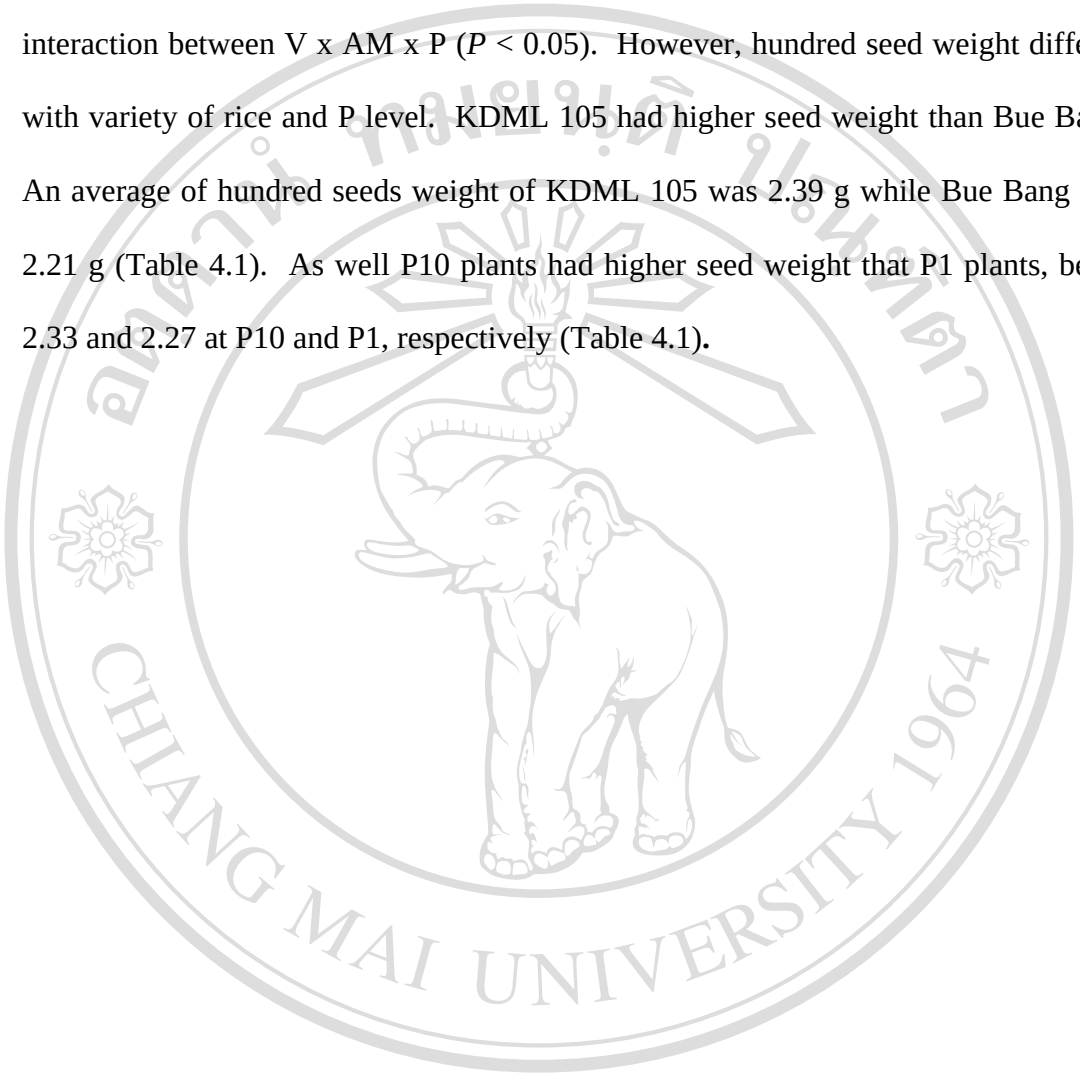
4.3.1 Vegetative growth

There was no Vx P x AM interaction for any growth parameters measured (plant height, shoot or root dry weight or seed weight) but there was a V x AM interaction for root dry weight (Figures 4.1-4.4). Inoculation had no effect on shoot or total dry weights but did affect plant height. Rice inoculated with mixed AM (AM2) were shorter than AM1 or AM0 plants (Figure 4.1). At nine weeks after sowing, KDML 105 and Bue Bang were 104.8 and 94.1 cm tall, respectively. Root dry weight of uninoculated KDML 105 (AM0) was lower than plants inoculated with AM1 or AM2 (Figure 4.3b). By contrast, root dry weight of Bue Bang did not differ with AM treatment. Lastly, phosphorus fertilization increased dry matter accumulation in both the shoots and roots (Figure 4.3a, b). Overall, dry matter accumulation in KDML 105 exceeded than in Bue Bang.

4.3.2 Reproductive growth

Panicle number was higher in KDML 105 than Bue Bang and increased with P supply (Figure 4.2). Total seed number and seed weight were increased by increasing P from P1 to P10 and KDML 105 had higher seeds number and seed weight than Bue Bang (Figure 4.4a, b). Seed weight of Bue Bang was lowest at P1 and at P10 was

equal to that of KDML 105 at P1 (V x P, $P < 0.05$). There was no effect of inoculation on seed weight or seed number. For hundred seed weight, there was no interaction between V x AM x P ($P < 0.05$). However, hundred seed weight differed with variety of rice and P level. KDML 105 had higher seed weight than Bue Bang. An average of hundred seeds weight of KDML 105 was 2.39 g while Bue Bang was 2.21 g (Table 4.1). As well P10 plants had higher seed weight that P1 plants, being 2.33 and 2.27 at P10 and P1, respectively (Table 4.1).



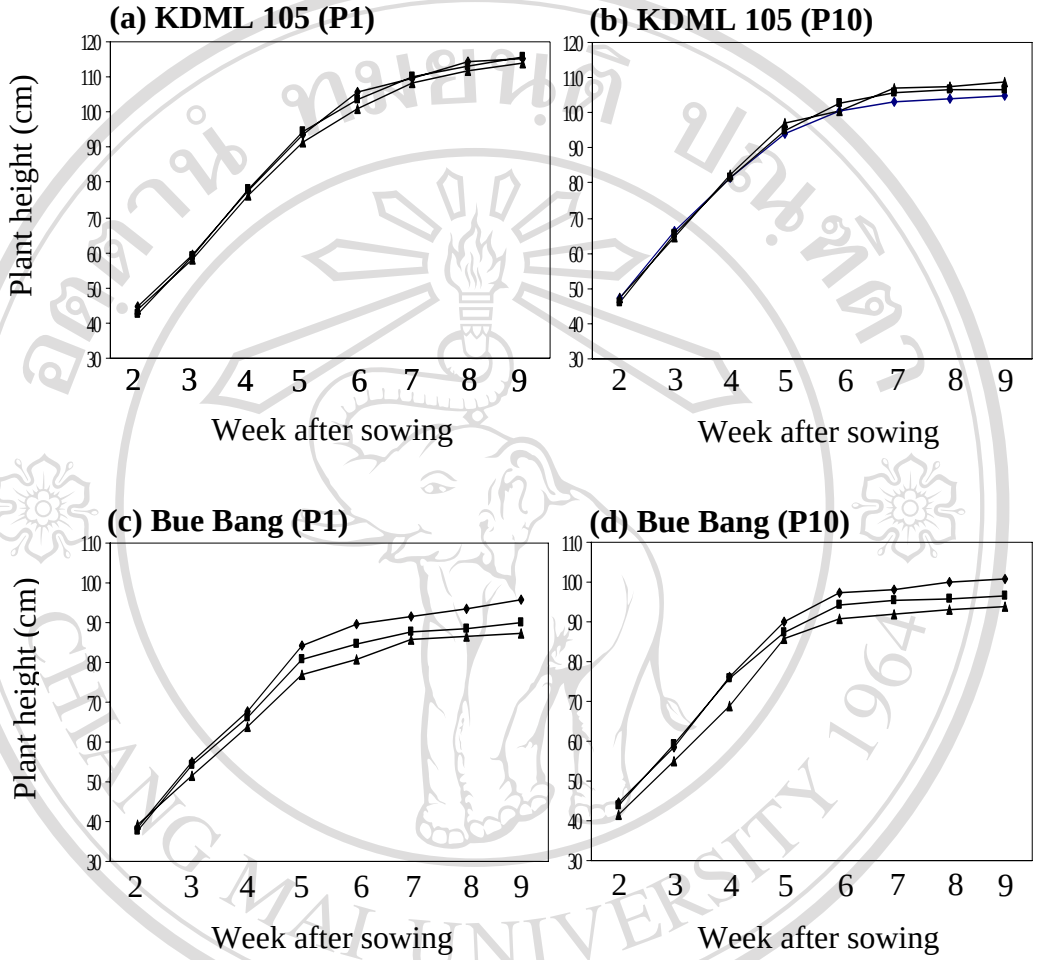


Figure 4.1 Height of lowland rice cv. KDML 105 and upland rice cv. Bue Bang from 2 weeks to 9 weeks as affected by AM inoculation (AM0:◆ , AM1:■ , AM2:▲) at two P levels (P1: 1 kg P ha^{-1}, P10: 10 kg P ha^{-1})

Analysis of variance for plant height at the ninth weeks

Analysis of variance	Effect						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
Plant height	**	**	ns	ns	ns	ns	ns

** significant at $P < 0.01$, ns = not significant at $P < 0.05$

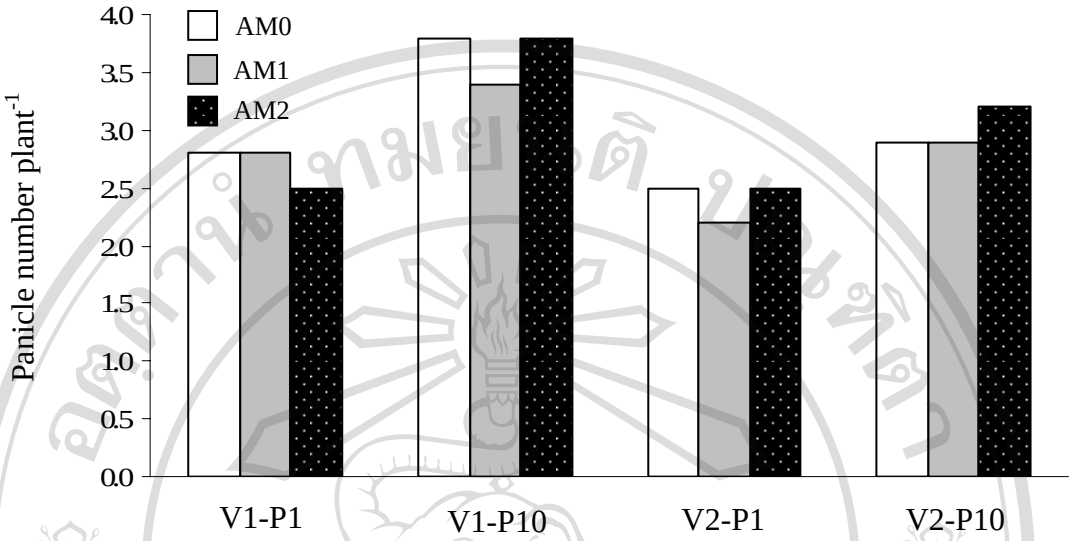


Figure 4.2 Panicle numbers of lowland rice cv. KDML 105 (V1) and upland rice cv. Bue Bang (V2) as affected by AM inoculation with two P level (P1: 1 kg P ha^{-1}, P10: 10 kg P ha^{-1}).

Analysis of variance for panicle numbers $plant^{-1}$

Analysis of variance	Effect						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
Panicle number	**	ns	**	ns	ns	ns	ns

** significant at $P < 0.01$, ns = not significant at $P < 0.05$

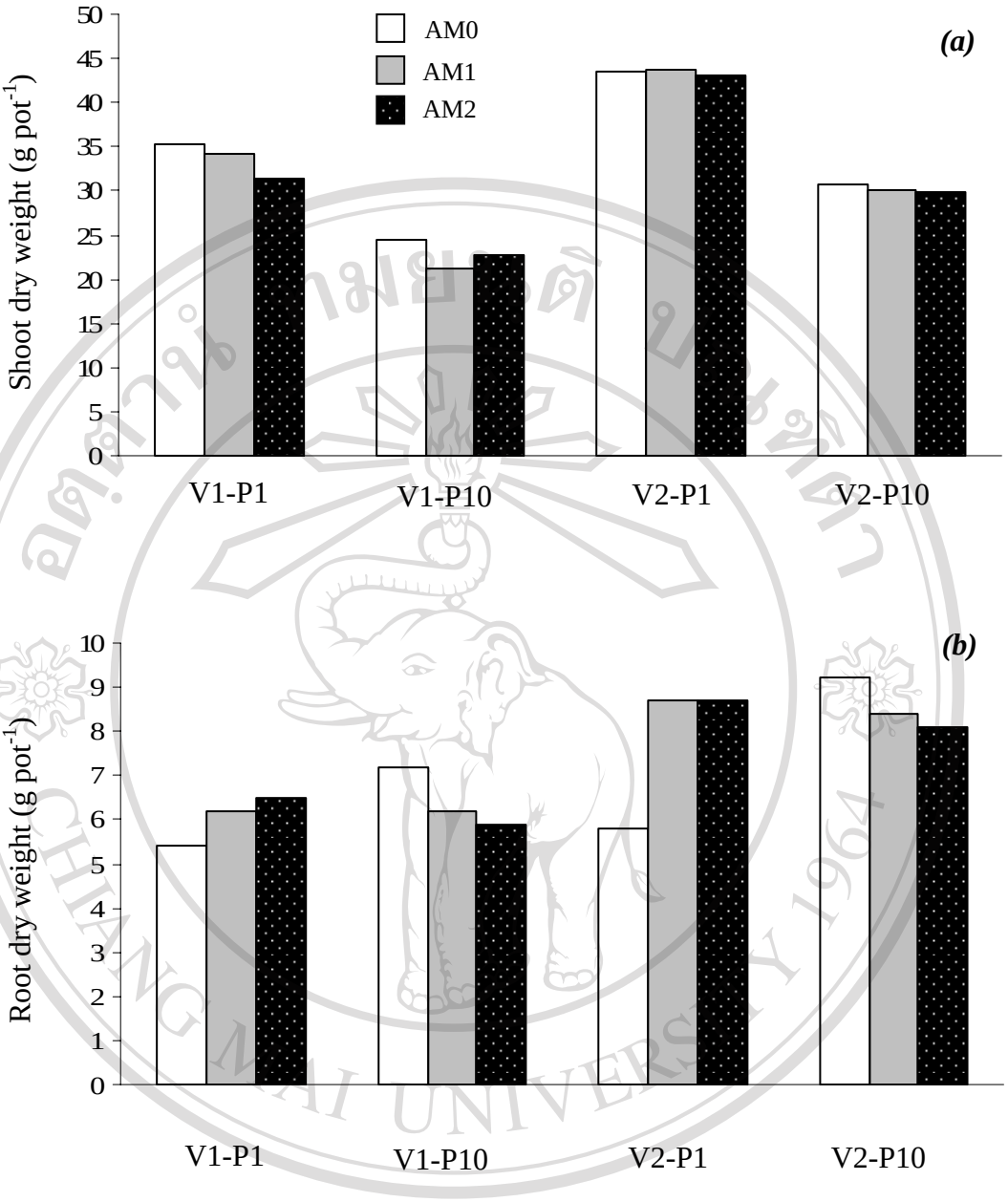


Figure 4.3 Shoot dry weight (a) and root dry weight (b) of lowland rice cv. KDML 105 (V1) and upland rice cv. Bue Bang (V2) as affected by AM inoculation with two P level (P1: 1 kg P ha^{-1}, P10: 10 kg P ha^{-1}).

Analysis of variance for shoot and root dry weight

Analysis of variance	Effect						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
Shoot dry weight	**	ns	**	ns	ns	ns	ns
Root dry weight	ns	ns	**	**	ns	ns	ns

** significant at $P < 0.01$, ns = not significant at $P < 0.05$

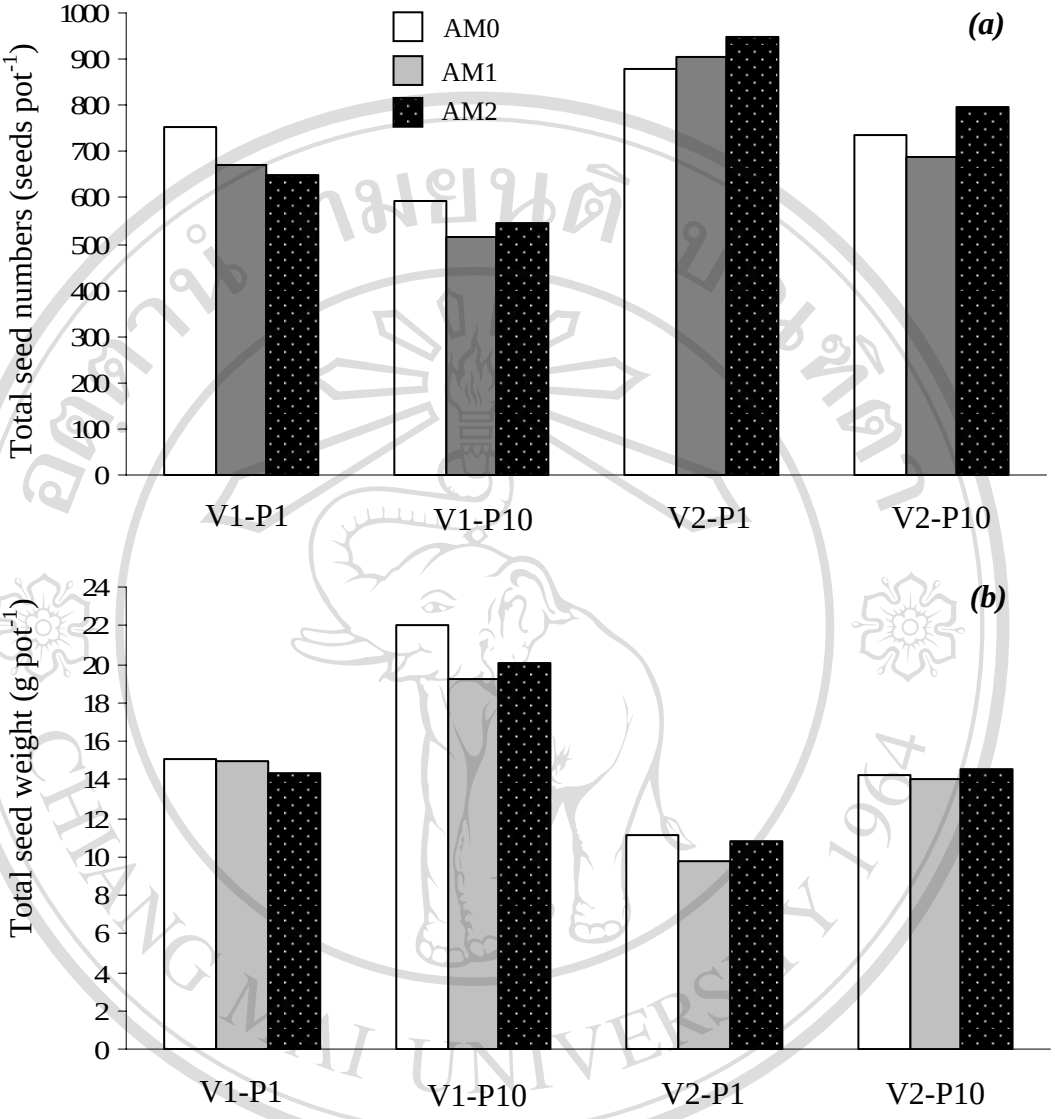


Figure 4.4 Total seed numbers (a) and total seed weight (b) of lowland rice cv. KDML 105 (V1) and upland rice cv. Bue Bang (V2) as affected by AM inoculation with two P levels (P1: 1 kg P ha^{-1}, P10: 10 kg P ha^{-1}).

Analysis of variance for shoot and root dry weight

Analysis of variance	Effect						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
Total seed numbers	**	ns	**	ns	ns	ns	ns
Total seed weight	**	ns	**	ns	*	ns	ns

* significant at $P < 0.05$, ** significant at $P < 0.01$, ns = not significant at $P < 0.05$

Table 4.1 Effects of AM inoculation and phosphorus application on hundred seed weight (g) of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	2.32	2.16	2.24
	AM1	2.41	2.23	2.32
	AM2	2.37	2.12	2.24
10	AM0	2.44	2.27	2.35
	AM1	2.36	2.26	2.31
	AM2	2.42	2.23	2.33
mean	P1	2.37	2.17	2.27 B
	P10	2.41	2.27	2.33 A
mean	AM0	2.38	2.22	2.30
	AM1	2.38	2.18	2.31
	AM2	2.40	2.25	2.29
mean	V	2.39A	2.21B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	ns	*	ns	ns	ns	ns

Different letters in each treatment indicate significant differences between a hundred seed weight by LSD at $P < 0.05$. * significant at $P < 0.05$, ** $P < 0.01$, ns = not significant $P < 0.05$.

4.3.3 Nutrient uptake

Nitrogen uptake of KDML 105 and Bue Bang did not differ with P or AM inoculation (Table 4.2). Mean N contents, averaged across all treatments, were 210 and 201.6 mg pot^{-1} for KDML 105 and Bue Bang, respectively (Table 4.2). By contrast, the cultivars differed in their P and K uptake and there was a response to P fertilizer for both elements (Tables 4.3, 4.4). The P content of KDML 105 (7.2 mg pot^{-1}) was higher than that of Bue Bang (5.9 mg pot^{-1}), and uptake was greater at P10 than at P1 (Table 4.3). For K uptake, rice varieties responded differently to AM inoculation (V x AM, $P < 0.05$) and P application (V x P, $P < 0.01$). Potassium uptake of Bue Bang did not differ between AM treatments, whereas KDML 105 uninoculated plants (AM0) had reduced K uptake (392.4 mg pot^{-1}) compared with plants inoculated with AM1 (457.1 mg pot^{-1}) or AM2 (456.7 mg pot^{-1}). However, K uptake of KDML 105 was higher than Bue Bang (Table 4.4).

Table 4.2 Effects of AM inoculation and phosphorus application on total nitrogen uptake (mg pot^{-1}) of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	215.4	211.0	213.2
	AM1	213.9	207.9	210.9
	AM2	221.7	190.2	205.9
10	AM0	190.0	216.1	203.0
	AM1	216.9	198.6	207.8
	AM2	202.1	186.0	194.0
mean	P1	217.0	203.0	210.0
	P10	203.0	200.2	201.6
mean	AM0	202.7	213.5	208.1
	AM1	215.4	203.2	209.3
	AM2	211.9	188.0	200.0
mean	V	210.0	201.6	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	ns	ns	ns	ns	ns	ns	ns

ns = not significant at $P < 0.05$.

Table 4.3 Effects of AM inoculation and phosphorus application on total phosphorus uptake (mg pot^{-1}) of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	5.8	5.0	5.4
	AM1	5.5	5.0	5.2
	AM2	7.0	5.4	6.2
10	AM0	7.6	5.8	6.7
	AM1	8.5	6.8	7.6
	AM2	8.8	7.3	8.0
mean	P1	6.1	5.1	5.6 B
	P10	8.3	6.7	7.5 A
mean	AM0	6.7	5.4	6.0
	AM1	7.0	5.9	6.4
	AM2	7.9	6.4	7.1
mean	V	7.2 A	5.9 B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	ns	**	ns	Ns	ns	ns

Different letters in each treatment indicate significant differences between shoot P uptake by LSD at $P < 0.05$. ** significant at $P < 0.01$, ns = not significant $P < 0.05$.

Table 4.4 Effects of AM inoculation and phosphorus application on total potassium uptake (mg pot^{-1}) of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	363.4	161.2	262.3
	AM1	366.7	142.0	254.4
	AM2	393.6	160.5	277.0
10	AM0	421.4	210.2	315.8
	AM1	547.4	194.2	370.8
	AM2	519.7	181.9	350.8
mean	P1	374.6b	154.6d	264.6
	P10	492.2a	195.5c	345.8
mean	AM0	392.4b	185.7c	289.1
	AM1	457.1a	168.1c	312.6
	AM2	456.7a	171.2c	313.9
mean	V	435.4 A	175.0 B	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	ns	**	*	**	ns	ns

Different letters indicate significant differences between shoot K uptake by LSD at $P < 0.05$. * significant at $P < 0.05$, **$P < 0.01$,
ns = not significant $P < 0.05$.

4.3.4 Nutrient concentration in brown rice and husk

4.3.4.1 Brown rice

Inoculation with AM fungi increased P and Cu concentrations in brown rice (Tables 4.6, 4.7). At both P levels, inoculation with AM2 enhanced the P concentration of both rice varieties. Inoculation with *Scutellospora* increased the P only at P1 (Table 4.6). Phosphorus concentration of both rice varieties was increased by 10.3% by AM2 inoculation. Brown rice of Bue Bang had a higher P concentration than KDML 105, 1530 and 1320 mg kg^{-1}, respectively. The grain P concentration at P10 was higher than at P1 (Table 4.6).

The Cu concentration was higher in seed of plants inoculated with AM2 than in treatments that were uninoculated (AM0) or inoculated with *Scutellospora* (AM1). Mixed AM increased Cu concentration in brown rice from 9.8 to 11.9 mg kg^{-1} in KDML 105 and from 11.8 to 15.8 mg kg^{-1} in Bue Bang (Table 4.7). Bue Bang had 29% higher Cu concentration than KDML 105 and application of high P depressed the Cu levels by up to 12.6% (Table 4.7).

However, N and Fe concentration in brown rice were lower in AM2 than AM0 plants. The N concentration was 1.75% when inoculated with AM2 whereas, inoculated with AM1 or uninoculated it was 1.84% and 1.89%, respectively (Table 4.8). The Fe concentration varied with inoculation treatment, being highest (18.5 mg kg^{-1}) at AM0 and lowest at AM1 (14.1 mg kg^{-1}). The AM2 treatment (16.7 mg kg^{-1}) was not significantly different from the other two treatments (Table 4.9).

Bue Bang had higher concentrations of P, K, S, Cl, Zn, Mn and Fe in brown rice than KDML 105 (Table 4.12). Magnesium, however, was higher in KDML 105 than in Bue Bang. Concentrations of N was similar in brown rice of both varieties

(Table 4.12). Inoculation did not alter the concentrations of K, S, Ca, Na, Mg, Cl, Zn or Mn in brown rice (appendix B), the concentration of Ca and Na was 0.02 and 0.01%, repectively (data not shown) and N, P, K, Ca, Cl, Na, Mg, Fe, Zn, Cu and Mn in husk (appendix C).

4.3.4.2 Husk

The main factor affecting nutrient concentration in the husk was rice variety (Table 4.10). Only sulfhur (S) showed an interaction between variety and AM inoculation (V x AM, $P < 0.01$). Unlike in KDML 105, the S concentration in Bue Bang was affected by AM treatment, being lowest in AM0 (0.078%) and increasing to 0.108% in AM1. Sulfur levels in AM2 were twice those in AM0 (Table 4.11). Generally, most nutrient concentration in the husk of Bue Bang were higher than KDML 105, the exception being the lower P and N concentration in Bue Bang. Lastly, there was no difference in Fe concentration with variety (Table 4.12).

Table 4.5 Analysis of variance for nutrients concentration in brow rice of two rice varieties

Analysis of variance	*F-test* of effects						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
%P	***	**	***	ns	ns	ns	ns
%N	ns	***	*	ns	**	ns	ns
%K	***	ns	ns	ns	ns	*	ns
%S	***	ns	ns	ns	ns	ns	ns
%Mg	***	ns	***	ns	ns	ns	ns
%Cl	*	ns	ns	ns	ns	ns	ns
Cu (mg kg^{-1})	***	***	***	ns	ns	ns	ns
Zn (mg kg^{-1})	***	ns	**	ns	ns	ns	ns
Mn (mg kg^{-1})	*	ns	*	ns	ns	ns	ns
Fe (mg kg^{-1})	**	*	ns	ns	ns	*	ns

* significant at $P < 0.05$

** significant at $P < 0.01$

*** significant at $P < 0.001$

ns = not significant at $P < 0.05$

Table 4.6 Effects of AM inoculation and phosphorus application on P concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	0.113	0.133	0.123
	AM1	0.123	0.150	0.136
	AM2	0.129	0.142	0.135
10	AM0	0.140	0.160	0.150
	AM1	0.138	0.159	0.148
	AM2	0.152	0.176	0.164
mean	P1	0.122	0.142	0.132 B
	P10	0.143	0.165	0.154 A
mean	AM0	0.126	0.146	0.136 B
	AM1	0.130	0.154	0.142 AB
	AM2	0.140	0.159	0.150 A
mean	V	0.132 B	0.153 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	**	**	ns	ns	ns	ns

Different letters indicate significant differences between P concentration by LSD at $P < 0.05$. ** significant at $P < 0.01$, ns = not significant $P < 0.05$.

Table 4.7 Effects of AM inoculation and phosphorus application on Cu concentration (mg kg^{-1}) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	10.3	11.8	11.0
	AM1	10.2	13.5	11.8
	AM2	13.1	16.2	14.6
10	AM0	9.4	11.9	10.6
	AM1	8.4	10.9	9.7
	AM2	10.7	15.4	13.1
mean	P1	11.2	13.8	12.5 A
	P10	9.5	12.7	11.1 B
mean	AM0	9.8	11.8	10.8 B
	AM1	9.3	12.2	10.8 B
	AM2	11.9	15.8	13.8 A
mean	V	10.3 B	13.3 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	**	**	ns	ns	ns	ns

Different letters indicate significant differences between Cu concentration by LSD at $P < 0.05$. ** significant at $P < 0.01$, ns = not significant $P < 0.05$.

Table 4.8 Effects of AM inoculation and phosphorus application on N concentration (%) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	1.95	1.83	1.89
	AM1	1.89	1.86	1.87
	AM2	1.85	1.74	1.80
10	AM0	1.88	1.90	1.89
	AM1	1.79	1.81	1.80
	AM2	1.66	1.75	1.70
mean	P1	1.89a	1.81b	1.85 A
	P10	1.77b	1.82b	1.79 B
mean	AM0	1.91	1.87	1.89 A
	AM1	1.84	1.83	1.84 A
	AM2	1.76	1.75	1.75 B
mean	V	1.83 A	1.81 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	ns	*	**	ns	**	ns	ns

Different letters indicate significant differences between P concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, $P < 0.01$, ns = not significant $P < 0.05$.

Table 4.9 Effects of AM inoculation and phosphorus application on Fe concentration (mg kg^{-1}) in brown rice of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
1	AM0	20.4	20.1	20.3 A
	AM1	12.7	16.6	14.6 B
	AM2	12.1	16.2	14.1 B
10	AM0	13.5	20.0	16.8 AB
	AM1	10.3	16.9	13.6 B
	AM2	16.6	22.2	19.4 A
mean	P1	15.1	17.6	16.3 A
	P10	13.4	19.7	16.6 A
mean	AM0	17.0	20.1	18.5 A
	AM1	11.5	16.8	14.1 B
	AM2	14.3	19.2	16.7 AB
mean	V	14.2 B	18.7 A	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	*	ns	ns	ns	*	ns

Different letters indicate significant differences between P concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, ** $P < 0.01$, ns = not significant $P < 0.05$.

Table 4.10 Analysis of variance for nutrients concentration in rice husk of two rice varieties

Analysis of variance	*F-test* of effects						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
%P	*	ns	ns	ns	ns	ns	ns
%N	**	ns	ns	ns	ns	ns	ns
%K	***	ns	*	ns	ns	ns	ns
%S	***	***	*	**	ns	ns	ns
%Na	ns	ns	ns	*	ns	ns	ns
%Ca	***	ns	*	ns	ns	ns	ns
%Mg	***	ns	***	ns	ns	ns	ns
%Cl	**	ns	ns	ns	ns	ns	ns
Cu (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
Zn (mg kg^{-1})	***	ns	ns	ns	ns	ns	ns
Mn (mg kg^{-1})	*	ns	ns	ns	ns	ns	ns
Fe (mg kg^{-1})	ns	ns	ns	ns	ns	ns	ns

* significant at $P < 0.05$

** significant at $P < 0.01$

*** significant at $P < 0.001$

ns = not significant at $P < 0.05$

Table 4.11 Effects of AM inoculation and phosphorus application on S concentration (%) in husk of two rice varieties

P level (kg ha^{-1})	Inoculation	KDML105	Bue Bang	mean
	AM0	0.049	0.077	0.063
1	AM1	0.055	0.121	0.088
	AM2	0.064	0.143	0.104
	AM0	0.049	0.079	0.064
10	AM1	0.054	0.094	0.074
	AM2	0.059	0.115	0.087
mean	P1	0.056	0.114	0.085 A
	P10	0.054	0.096	0.075 B
mean	AM0	0.049d	0.078c	0.064
	AM1	0.054d	0.108b	0.081
	AM2	0.062d	0.129a	0.095
mean	V	0.055	0.105	

Effect	V	AM	P	VxAM	VxP	AMxP	VxAMxP
F-test	**	**	*	**	ns	ns	ns

Different letters indicate significant differences between S concentration by LSD at $P < 0.05$. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

Table 4.12 Comparison of nutrient concentration in brown rice and husk of two rice varieties (B = Bue Bang, K = KDML 105)

Nutrients concentration	Part of seed	
	Brown rice	Husk
P (%)	B>K	K>B
N (%)	ns	K>B
K (%)	B>K	B>K
S (%)	B>K	B>K
Na (%)	ns	ns
Ca (%)	ns	B>K
Mg (%)	K>B	B>K
Cl (%)	B>K	B>K
Cu (mg kg^{-1})	B>K	B>K
Zn (mg kg^{-1})	B>K	B>K
Mn (mg kg^{-1})	B>K	B>K
Fe (mg kg^{-1})	B>K	ns

ns = no significant at $P < 0.05$

4.3.5 **AM infection and spore density**

The extent of root colonization by AM fungi was affected by P and inoculation (P x AM, $P < 0.05$). The percentage root colonization of the two cultivars was similar (over 90%, Figure 4.5) when inoculated with mixed AM fungi (AM2) and there was no effect of P fertilizer. However, with *Scutellospora* (AM1) root infection was much lower at 30-40% in P1 and depressed to about 10% in P10 (Figure 4.5). There was no infection in the control plants inoculated with autoclaved soil.

Spore density of AM fungi varied with rice variety, AM inoculation and P level (V x AM x P, $P < 0.05$). At both P levels, Bue Bang had higher AM2 spores counts than KDML 105. However, increasing P from P1 to P10 strongly depressed AM2 spores of Bue Bang by 3 times. Whereas, AM2 spore of KDML 105 was lowest. Few spore of AM2 were produced in the rhizosphere of KDML 105 (3 and 1 spore g^{-1} soil at P1 and P10, respectively. Adding P did not depress AM1 spore number in either KDML 105 or Bue Bang. The greatest spore number were found in Bue Bang when inoculated with AM2 (32 spore g^{-1} soil).

Based on morphology, *Acaulospora* was the dominant genus of AM fungi spores in AM2, the mixed spore inoculum. However, there were also some differences due to rice variety and P fertilizer. *Acaulospora* accounted for almost all of the spores in both rice varieties except for KDML 105 at P10 where *Glomus* became the dominant genus (Figure 4.6). At the same soil P level, the percentage of each spore type differed with rice variety (Figure 4.6).

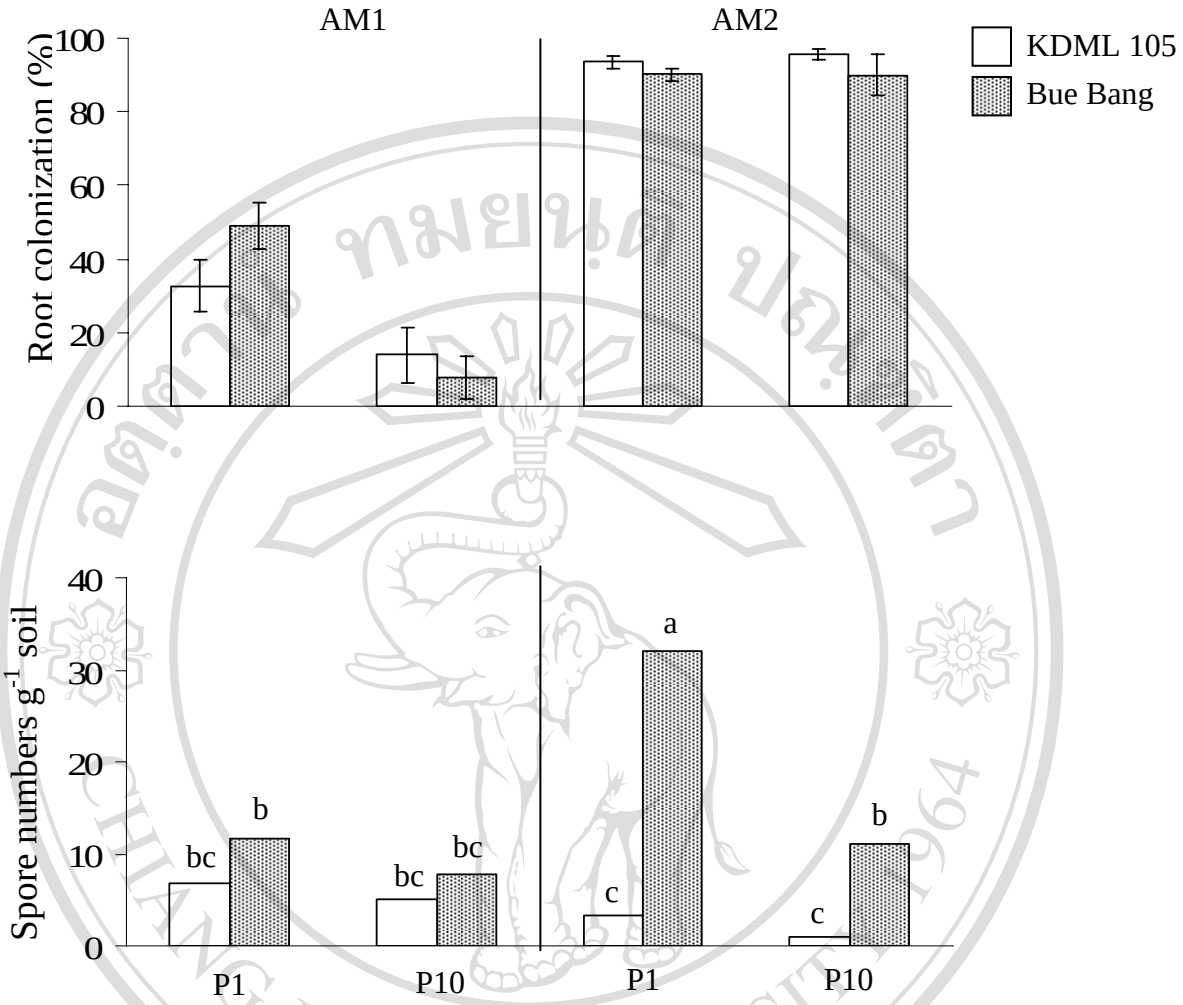


Figure 4.5 The percentage of root colonization and spore density of two rice varieties (KDML 105 and Bue Bang) with two AM inoculation (AM1: *Scutellospora* spores and AM2: mixed AM spores) at two P levels (P1: 1 kg P ha^{-1} and P10: 10 kg P ha^{-1}). The vertical bar at the percentage of root colonization represented ± S.E. The same letter above bar of spore number g^{-1} soil were not significant at $P < 0.05$.

Analysis of variance (after arcsine transformation for root colonization)

Analysis of variance	Effect						
	V	AM	P	VxAM	VxP	AMxP	VxAMxP
Root colonization	ns	**	*	ns	ns	**	ns
Spore density	**	*	**	**	*	*	*

* significant at $P < 0.05$, ** significant at $P < 0.01$, ns = not significant at $P < 0.05$

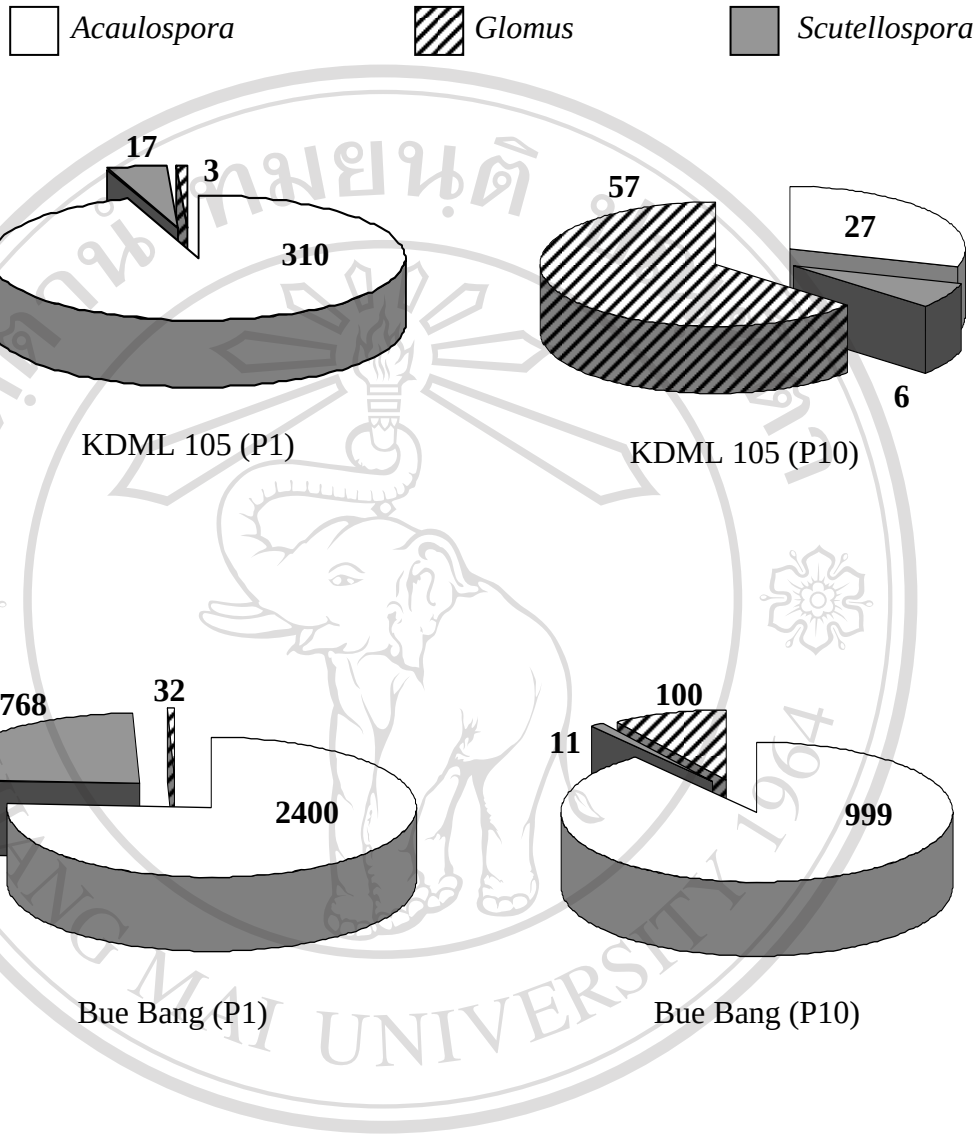


Figure 4.6 Effect of rice variety on AM fungi spore type that developed from a soil inoculant of mixed spores types at two P levels. The pie sections indicate portion of the different genera, with number of spores of each genus 100 g^{-1} soil.

4.4 Discussion

Even though the roots were heavily colonised by arbuscular mycorrhizal fungi, vegetative growth of the two rice varieties was not promoted by the addition of live soil inoculum containing AM fungi spores nor by inoculation with pot-grown spores of one AMF species. There are a number of possibilities for this. Firstly, the AMF species, although they were compatible with rice roots, may be inefficient in acquiring and transferring nutrients such as P or other chemicals to the host plants to promote early growth. Secondly, rice may have low mycorrhizal dependency on AM fungi for its growth in acid soils. Hetrict *et al*. (1992) suggested/showed that the roots of plant species with high mycorrhizal dependency often have thick, unbranched roots, and few root hairs. By contrast, rice roots are fine, highly branched and have numerous root hairs, so mycorrhizal dependency of this plant could be low compared to pada (Chapter 3).

Although inoculation did not enhance vegetative growth in this study, it enhanced nutrient concentrations of some elements in reproductive tissues. Inoculation increased P and Cu concentrations in brown rice of both varieties. Moreover, its also increased S in the husk of Bue Bang. Although AM fungi enhanced the P concentration in brown rice of both KDML 105 and Bue Bang, the P concentration as higher in brown rice than KDML 105 (Table 4.6). At P1, both inoculation treatments increased the P concentration in brown rice of both rice varieties when compared with uninoculated plants, but at P10, only AM2 promoted P concentration in brown rice.

Changing soil P levels can markedly alter the role of AM to the host plant. Phosphorus added to the grown medium can inhibit hyphal branching directly in

addition to the quantity or quality of root exudates, regulated by host P status also affects hyphal branching (Nagahashi *et al*., 1996). At P1, AM fungi could increased P concentration in brown rice were between 0.113-0.129% and 0.133-0.150% and at P10 were between 0.138-0.152% and 0.159-0.176% in brown rice of KDML 105 and Bue Bang, respectively. From this experiment showed that AM fungi could enhanced P concentration and also Cu in brown rice when compared with uninoculated plants. Because of P is the important element as a component of high energy substrate (ATP) and sugar phosphate substrates (Marchner, 1995). So that better of nutrients concentration in brown rice in mycorrhizal plant is interest and was a good signal for improving seed nutrient quality.

Phosphorus application strongly affected to spore density more than percentage of root colonization. However, considered only percentage of root infection found that no effect of P added to colonization of root by AM2 (mixed AM spores). Colonization of root was over 90% at both P levels and both of rice varieties whereas, root colonization by AM1 (*Scutellospora*) was depressed by increased P to P10 (Figure 4.5). From that point, it was suggested that different AM types have differed for suffering of P application to soils grow medium. Variety of plant also have role on root infection, it was confirmed by different of degree root colonization by AM1 that was 33% in KDML 105 and 49% in Bue Bang. Spore number g^{-1} soil of AM1 and AM2 from rhizosphere of Bue Bang was higher than from KDML 105. At both P levels, the AM2 spores of KDML 105 was only 10% of spore of Bue Bang (Figure 4.5). However, spore number was declined by 3 times when applied P to P10.

Mixed AM fungi were identified on basis of spore morphology under compound- microscope and have used guidance from Youpensuk thesis (2004). It

was found as 3 genera: *Acaulospora*, *Glomus* and *Scutellospora* (Figure 4.6). Surprisingly, *Acaulospora* genus was a dominant genus in both rice rhizosphere and also P levels but at P10, *Glomus* became dominant genus in the rhizosphere of KDML 105. From this study, it was suggested that all host plant, AM types and P in soil affect AM production. From previous study found AM fungi increased biomass of *Macaranga denticulata* (pada). The greatest shoot N, P and K contents occurred in pada plants inoculated with *Acaulospora* spp. and from these result confirmed that *Acaulospora* spp. was an effective genus for growth of pada plant. From this study, *Acaulospora* was dominant genus found in root zones of plants inoculated with mixed AM species of AM fungi, indicating the better sporulating. This genus may be a key role to increased nutrient concentration in rice seed. However, it need to be confirmed by single culture.