CHAPTER 3

ASSOCIATIONS BETWEEN ARBUSCULAR MYCORRHIZAL FUNGI AND SWIDDEN CROPS

3.1 Introduction

Previous studies have shown the importance of arbuscular mycorrhizal (AM) fungi in nutrient accumulation and soil fertility maintenance role of a fallow enriching tree, pada (*Macaranga denticulata*), and the contribution of pada to upland rice yield in shifting cultivation at Huai Tee Cha village (Yimyam, 2006; Youpensuk, 2004). The farmers have maintained their livelihood with traditional shifting cultivation or swidden agriculture. Most people are very poor, using no input of fertilizer or lime to improve soil fertility, thus yield from the upland cropping system is dependent on native quality of the soil. The previous field's study of Huai Tee Cha village (Chapter 2), has found that swidden crops in the farmers' field at Tee Cha to be infected by AM fungi. A question is raised on how much the AM fungi contribute to upland rice and other food crops in the system directly, in addition to the effect through nutrient accumulation by pada. The purposes of this study were to examine the association of upland rice and other food crops with AM fungi from the farmers' fields and to evaluate the role of AM fungi on growth, yield and nutrient uptake in these food crops experimentally. Finding out how food crops respond to the AM fungi would be useful to other farmers who operate similar and other systems of crop production on soils of low fertility in this and other countries.

3.2 Materials and Methods

Experimental design

The experiment was conducted in CRD with factorial combination of 2 levels of P [3 kg P ha^{-1} (P3) or 30 kg P ha^{-1} (P30)] and 2 inoculation treatments [25 g plant^{-1} of soil inoculum (from Huai Tee Cha village as described by Youpensuk *et al*., 2004) containing ca. 5 spores g^{-1} (AM+), or soil inoculum that had been autoclaved at 121 °C for 60 minutes (AM0)], with three replicate pots. The two P treatments were chosen to represent Tee Cha soils of low (P3) and moderate [P30, ca. 70% maximum yield of uninoculated pada (Youpensuk *et al*., 2004)] P availability. Soil containing AM fungi spores was chosen as inoculum rather than extracted spores as this is the inoculum form that is available to farmers at Huai Tee Cha village should they choose to amend poorer fields in the future. The spore composition of the inoculum was comprised of *Acaulospora, Glomus, Scutellospora* and others (*Archaeospora, Gigaspora, Paraglomus*) in the proportion 4:3:2:1. The experiment was conducted in an outdoor screen house consisting of a plastic roof and mesh walls, from August (rainy season) to December (cool season) 2005, with average max/min temperature of 30/21 °C, at the Agronomy Department, Chiang Mai University.

3.2.1 Seed and soil preparation

Seed of the four swidden crops, that are normally grown after slashing and burning the regenerated forest, including Job's tears (*Coix lachryma-jobi* L.), corn (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and upland rice cv *Bue Bang* (*Oryza sativa* L.) and seed of the fallow enriching tree, pada (*Macaranga denticulata* (Bl.) Muell. Arg) were provided by farmers in Huai Tee Cha village. All crops seed were tested for germination in order to estimate the number of seeds required per planting hole (3 holes pot^{-1}). The seeds were surface sterilized in 70% ethanol for five minutes and washed five times with sterile water. The soil inoculum, 25 g $planting^{-1}$ hole, was placed under the seed in plastic pots (21 cm top diameter, 14 cm bottom diameter, and 16 cm depth) with basal drainage holes containing a mixture of Sansai soil (1.6 mg kg^{-1} Bray II P, pH 5.6) and coarse river sand (2:1 v/v) that had been steam-pasteurized at 95 °C for five hours and repeated after 24 hrs. The soil has a sandy loam texture, belongs to the Tropaqualf of Sansai soil series and was chosen because it has very low available P and had been used previously in mycorrhizal pot trials with pada (Youpensuk *et al*., 2004). Cultural sensitivities precluded the removal of soil from Huai Tee Cha village for this trial. Phosphorus was added as finely ground triple superphosphate and mixed throughout the dry soil before planting. Each pot received the following total basal nutrients on a surface area basis: 100 kg N ha^{-1} as urea and 50 kg K ha^{-1} as KCl. Nitrogen and K were applied in solution onto the soil surface every two weeks. Other basal nutrients were not applied as there had been no previous reports of deficiencies other than N, P and K in the Sansai soil. 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.

3.2.2 Harvest

Crops were harvested two times, mostly when there were visible differences due to inoculation at the vegetative stage (H1) and at maturity (H2). Harvest times were 7 weeks and 17 weeks in Job’s tears and sorghum; 8 weeks and 12 weeks in corn. Upland rice which showed no visible effect of inoculation was harvested at maximum tillering (9 weeks) and maturity (17 weeks). Pada was harvested once, at 20 weeks after sowing.

Prior to the final 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). At each harvest, the shoot was partitioned into stems, leaves, seeds (at maturity) and the roots were washed free of soil. The roots were subsampled for determining root colonization (Figure 3.1) and examination of spores as described in 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.

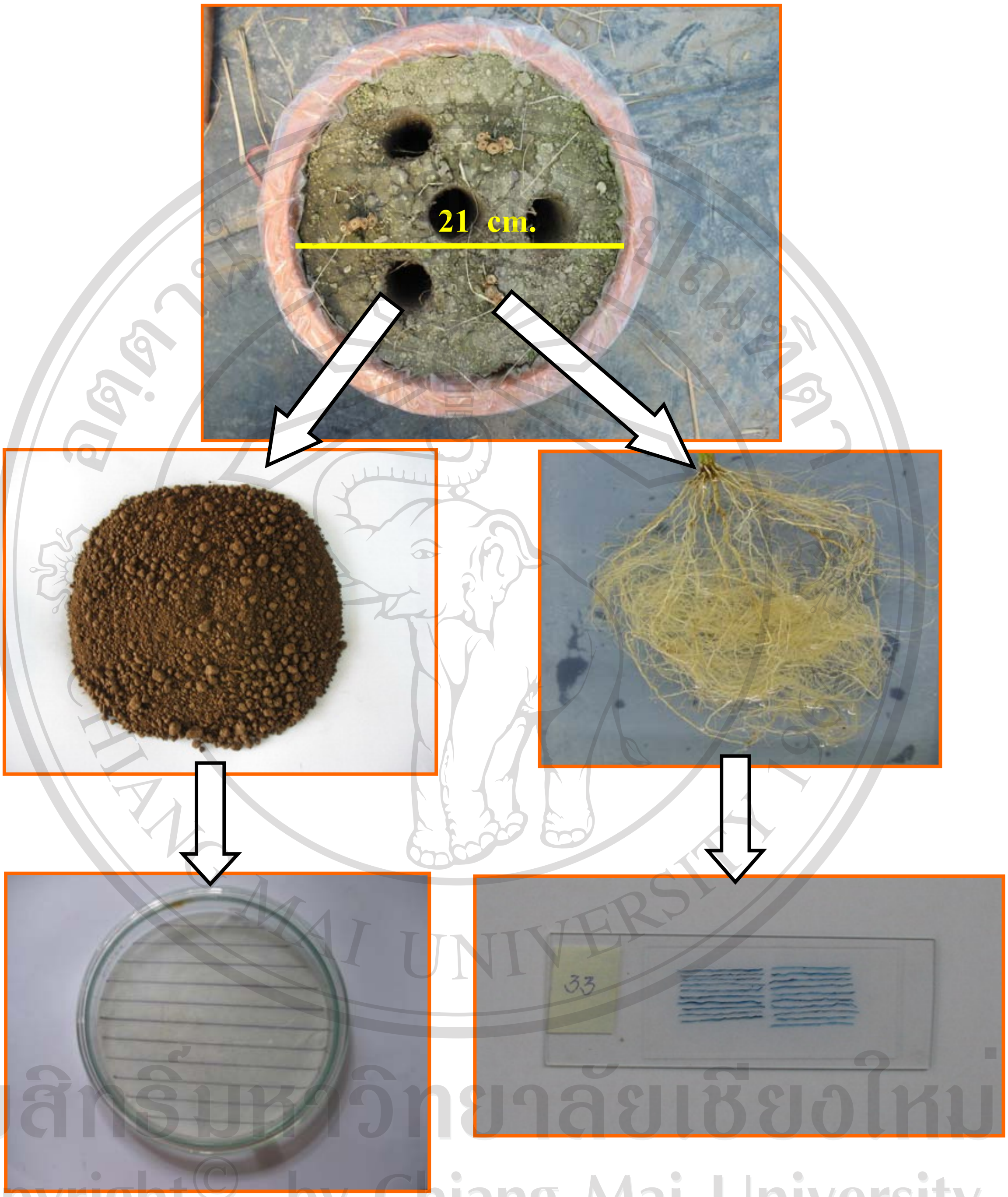


Figure 3.1 The procedure of soil and root samples collection for determination of spore density under stereoscope (Petri dish) and percentage of root colonization under compound microscope (Slide).

3.2.3 Mycorrhizal responsiveness (MR) for growth and nutrient uptake

Mycorrhizal growth responsiveness (MGR) or mycorrhizal dependency (MD) was calculated after Plenchette *et al*. (1983): %MGR = [Dry mass (AM+) – Dry mass (AM0)]/ Dry mass (AM+) x 100; and mycorrhizal phosphorus responsiveness (MPR) was calculated as follows: %MPR = [P content (AM+) – P content (AM0)]/ P content (AM+) x 100, where AM+ = inoculated plants and AM0 = uninoculated plants. Responses to AM fungi were determined in several level of P fertilizer, for this reason, the percentage of all mycorrhizal responsiveness is suitable specific each P level. For this experiment: mycorrhizal responsiveness at P3 or P30 is defined by $\%MR_{P3}$ and $\%MR_{P30}$, respectively.

3.2.4 Data analysis

Data were analyzed by using commercial software (Statistix V. 8, Analytical Software, Inc.). Total dry weight, nutrient uptake and seed weight were log transformed and analysed by analysis of variance (ANOVA) separately for plant species and harvest. Percentage data for root colonization was arcsine transformed and spore density was log transformed before analysis. Least significant difference (LSD) at 5% confidence level was used for comparison under ANOVA.

3.3 Results

3.3.1 Plant dry weight

3.3.1.1 Shoot dry weight

Responses to AM+ inoculation began to be obserbed in dry weights in corn and Job’s tears at H1 and continued to H2 when the response in those also became significant and pada which was harvested only once, at about the same time as H2 in the crops. There was a significant interaction between P and inoculation (Table 3.1) for shoot dry weight of corn and Job’s tears (H1, 2), sorghum (H2) and pada (H2). Dry weight of upland rice in both harvest, however did not respond to AM+ inoculation, although its dry weight was significanttly increased with the increase in P application. The effect of AM+ inoculation on dry weight of those responsive species was strongly dependent on the level of P application (AM x P significant at $P < 0.05$). The strongest response to AM inoculation was found in pada, in which shoot biomass was increased 40 times by AM+ inoculation at P3, to the same extent as and application of 30 kg P ha^{-1}. A similar but smaller response occurred in corn, Job’s tears and sorghum, AM effect was half of that of 30 kg P ha^{-1}. All plants responded to the addition of P alone: the greatest increase was in pada and the smallest in upland rice. None of the species showed dry weight response to AM+ inoculation with 30 kg P ha^{-1} (Table 3.1)

3.3.1.2 Root dry weight

Except for a significant interaction between P and inoculation for root dry weight of Job’s tears at the first harvest, root biomass responses generally (Table 3.2) paralleled responses in shoot dry weight (Table 3.1). However, unlike for shoots, AM+ inoculation increased root dry weight of Job’s tears at both P3 and P30. The response in root dry weight of crops to AM+ inoculation at P3 ranged from a factor of 2.7 in sorghum to 3.3 in Job’s tears, and these were considerably lower than in pada (51x).

Table 3.1 Effects of phosphorus application and AM inoculation on shoot dry weight (g pot^{-1}) of four swidden crops and pada at two harvests

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
	Harvest 1 (H1)			
Corn	4.99c	9.33b	19.87a	23.10a
Job's tears	1.89c	3.89b	10.61a	13.11a
Sorghum	1.07	1.52	11.9	12.96
Upland rice	6.66	5.95	18.32	19.23
	Harvest 2 (H2)			
Corn	5.47c	10.85b	20.72a	24.89a
Job's tears	5.33c	12.37b	24.89a	31.29a
Sorghum	4.09c	13.79b	31.04a	34.85a
Upland rice	10.17	11.65	29.46	25.46
Pada	0.26b	10.40a	8.48a	13.30a

Analysis of variance F-test

Effect	Corn		Job's tears		Sorghum		Upland rice		Pada
	H1	H2	H1	H2	H1	H2	H1	H2	H2
AM	**	**	**	**	ns	**	ns	ns	**
P	*	**	*	*	**	**	**	**	**
AM x P	*	*	*	*	ns	**	ns	ns	**

Different letters in each row indicate significant differences between shoot dry weight by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

Table 3.2 Effects of phosphorus application and AM inoculation on root dry weight (g pot^{-1}) of four swidden crops and pada at two harvests

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
		Harvest 1 (H1)		
Corn	0.75c	1.85b	3.00a	3.03a
Job's tears	0.44d	1.07c	2.10b	2.56a
Sorghum	0.29	0.32	3.15	3.90
Upland rice	4.00	5.30	10.75	12.08
		Harvest 2 (H2)		
Corn	1.55c	2.63b	3.30ab	3.56a
Job's tears	1.39b	4.63a	4.76a	6.19a
Sorghum	0.91c	4.43b	5.84a	5.53ab
Upland rice	4.12	4.35	9.23	11.13
Pada	0.13b	6.72a	6.09a	6.59a

Analysis of variance F-test

Effect	Corn		Job's tears		Sorghum		Upland rice		Pada
	H1	H2	H1	H2	H1	H2	H1	H2	H2
AM	**	**	**	**	ns	**	ns	ns	**
P	**	*	*	**	**	**	**	**	**
AM x P	*	*	ns	**	ns	**	ns	ns	**

Different letters in each row indicate significant differences between root dry weight by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

3.3.2 Seed yield

AM+ inoculation increased seed weight of all three crops that produced seed: upland rice, sorghum and Job's tears (Table 3.3). Inoculation increased seed yield by 1.7 times in upland rice and 13 times sorghum at P3 ($P < 0.05$). These increases were 50% and 11%, respecively, of yield in P30 plants but there was no effect of AM+ at P30. Total seed per pot of upland rice did not differ between AM0 and AM+ plants at P3 wherase, AM0 plants have lower filled grain (67) than AM+ plants (133). In Job's tears seed yield was very small in AM0 and AM+ plants at P3 but AM+ inoculation still continued to increase its seed yield by almost 74% at P30. Of the three crops where grain was formed, reproductive yield of upland rice was the least affected by the low P supply. Also, grain yield was more sensitive to low P than vegetative yield in sorghum in the inoculated treatment. Pada and corn did not produce any seed by the time the experiment was terminated.

Table 3.3 Effects of phosphorus application and AM inoculation on seed yield (g pot^{-1}) of Job’s tear, sorghum and upland rice at maturity

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
Job’s tears	0.1d	0.5c	4.1b	7.3a
Sorghum	0.1c	1.3b	11.8a	11.7a
Upland rice	2.4c	4.0b	8.4a	8.5a
†Grain pot^{-1}	237.7	247.7	495.0	560.0
†Filled grain pot^{-1}	67.7c	133.3b	282.0a	300.0a

Analysis of variance

Effect	F-test				
	Job’s tears	Sorghum	Upland rice	†Grain pot^{-1}	†Filled grain pot^{-1}
AM	**	*	*	ns	*
P	**	**	**	**	**
AM x P	ns	**	*	ns	*

For same plant species, different letters are significantly different between seed weight by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

† data of upland rice

3.3.3 Nutrient uptake

3.3.3.1 Nitrogen uptake (N)

The effects of AM+ inoculation and P application on the uptake of nitrogen (N) depended on the species of host plants and the harvest. Accumulation of N nutrient was enhanced in AM+ plants by H1 in corn and Job's tears but not sorghum and upland rice (Table 3.4). In the first two crops, the effect continued to H2, AM+ increased N accumulation 2.1 times (H1) and 1.8 times (H2) in corn and 2.1 times (H1) and 1.7 times (H2) in Job's tears. By H2, the N uptake in sorghum and upland rice was increased by AM+ inoculation 2.4 and 1.3 times, respectively (Table 3.4). Nitrogen uptake of sorghum and upland rice at the first harvest was not affected by inoculation. However, increasing P application alone increased nitrogen uptake by all crop species (Table 3.4). In pada, AM+ had the same effect in increasing the amount of N in the plant as the increased in P application to P30. In contrast, N uptake in pada was about the same when AM and P30 were applied together or separately. In AM0 pada at P3, there was not enough sample for analysis, and based on the dry weight produced only about 5-6 mg N pot^{-1} was estimated to have been taken up (Table 3.4).

3.3.3.2 Phosphorus uptake (P)

There was an interaction between inoculation and P application on total phosphorus (P) uptake of corn (H1), Job's tears (H1), sorghum (H1), upland rice (H2) and pada. AM+ inoculation increased P uptake at P3 in both harvest of corn, Job's tears and sorghum. Inoculation increased P uptake at the H1 were 2.2, 2.7 and 2.2 times and at the H2 were 2.0, 2.0 and 2.6 times in corn, Job's tears and sorghum, respectively. Moreover, P accumulation was increased by 1.3 times at AM+ P30 in sorghum. Phosphorus uptake of upland rice did not increase by AM+ inoculation at the H1, however, AM+ increased P accumulation at the H2 by 1.5 times. Accumulation P of pada was increased in AM+ inoculation by 95 times at P3 and by 3 times at P30, AM+ had the same effect in increasing the amount of P in the plant as the increase in P application to P30. When AM+ was provided together with P30 to pada, however, the P uptake almost tripled that from P30 or AM+ alone (Table 3.5).

3.3.3.3 Potassium uptake (K)

There was also an interaction between inoculation and P application for potassium (K) uptake. AM+ increased K uptake in Job's tears and sorghum, especially at P3 whereas at P30, AM+ did not enhance K uptake. Inoculation had no effect on K accumulation in corn at both P3 and P30 (Table 3.6). By contrast, the K uptake of upland rice did not respond to AM+ at P3 but it was significant only at P30 in H1. In pada, the K uptake in the very small at AM0 plants at P3 was only 6 mg K pot^{-1} compared with more than 1,400 mg K pot^{-1} taken up with AM+, in either P3 or P30. At the H2, K uptake was higher in inoculated Job's tears and pada than uninoculated plants at both P rates. Increasing P alone increased K uptake of all plant species (Table 3.6).

Table 3.4 Effects of phosphorus application and AM inoculation on total nitrogen uptake (mg pot^{-1}) of four swidden crops at two harvests and pada

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
	Harvest 1 (H1)			
Corn	98.3b	177.3a	239.4a	240.6a
Job’s tears	64.6c	137.3b	257.1a	285.7a
Sorghum	30.6	44.9	247.1	225.7
Upland rice	167.9	199.7	379.1	387.3
	Harvest 2 (H2)			
Corn	141.3b	233.5a	253.1a	280.4a
Job’s tears	135.6c	235.2b	367.0a	363.3a
Sorghum	99.4c	239.6b	405.5a	324.5ab
Upland rice	184.2c	239.2b	432.4a	384.1a
Pada	nd	242.4a	225.1a	281.1a

Analysis of variance F-test

Effect	Corn		Job’s tears		Sorghum		Upland rice		Pada
	H1	H2	H1	H2	H1	H2	H1	H2	H2
AM	*	*	**	*	ns	*	ns	ns	**
P	**	**	**	**	**	**	**	**	**
AM x P	*	*	**	*	ns	**	ns	**	**

Different letters in each row indicate significant differences between N uptake by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

nd = not determined (not enough sample for determination), based on the dry weight produced about 5-6 mg N pot^{-1}

Table 3.5 Effects of phosphorus application and AM inoculation on total phosphorus uptake (mg pot^{-1}) of four swidden crops at two harvests and pada

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
		Harvest 1 (H1)		
Corn	2.6d	5.8c	14.5b	22.7a
Job’s tears	2.5c	6.8b	20.2a	23.7a
Sorghum	1.4c	3.1b	24.4a	25.1a
Upland rice	4.6	5.8	24.6	26.4
		Harvest 2 (H2)		
Corn	4.0c	8.2b	21.6a	25.0a
Job’s tears	5.4d	10.6c	43.4b	59.6a
Sorghum	2.8d	7.2c	28.4b	37.5a
Upland rice	6.8c	10.4b	37.9a	37.9a
Pada	0.1c	9.5b	8.4b	25.1a

Analysis of variance F-test

Effect	Corn		Job’s tears		Sorghum		Upland rice		Pada
	H1	H2	H1	H2	H1	H2	H1	H2	H2
AM	**	**	**	*	**	*	ns	**	**
P	**	**	**	**	**	**	**	**	**
AM x P	ns	*	**	ns	**	ns	ns	**	*

Different letters in each row indicate significant differences between P uptake by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

Table 3.6 Effects of phosphorus application and AM inoculation on total potassium uptake (mg pot^{-1}) of four swidden crops at two harvests and pada

Plant species	P3 (3 kg P ha^{-1})		P30 (30 kg P ha^{-1})	
	AM0	AM+	AM0	AM+
		Harvest 1 (H1)		
Corn	245.7	396.1	488.0	561.9
Job’s tears	77.5c	234.6 b	401.7a	408.5a
Sorghum	132.6c	201.9b	1214.4a	1235.8a
Upland rice	196.0c	216.3c	505.5b	704.2a
		Harvest 2 (H2)		
Corn	437.4	638.7	840.4	782.6
Job’s tears	358.8d	861.1c	1056.6b	1375.9a
Sorghum	227.3c	731.7b	1179.7a	990.8a
Upland rice	567.2	578.6	1395.0	1364.4
Pada	5.7b	1421.2a	1161.7a	1760.3a

Analysis of variance F-test

Effect	Corn		Job’s tears		Sorghum		Upland rice		Pada
	H1	H2	H1	H2	H1	H2	H1	H2	H2
AM	ns	ns	*	*	**	ns	*	ns	**
P	**	*	**	**	**	**	**	**	**
AM x P	ns	ns	*	ns	*	**	*	ns	**

Different letters in each row indicate significant differences between K uptake by LSD at $P < 0.05$ after log transformation. * significant at $P < 0.05$, **$P < 0.01$, ns = not significant $P < 0.05$.

3.3.4 Seed nutrient concentrations

Phosphorus fertilizer increased P concentrations in the whole seed (brown rice with husk) of Job's tears, sorghum and upland rice. However, AM+ enhanced ($P < 0.05$) P accumulation only in the whole seed of Job's tears and sorghum at P30. There were no effects of AM inoculation and P level on N concentration of any crop seeds. At P30, the whole seed K concentration of sorghum was lower than at P3 and was not affected by AM inoculation. There was interaction ($P < 0.05$) between AM and P for K concentration in the whole seed of upland rice due to a reduction in K in AM0 P3 plants (Figure 3.2).

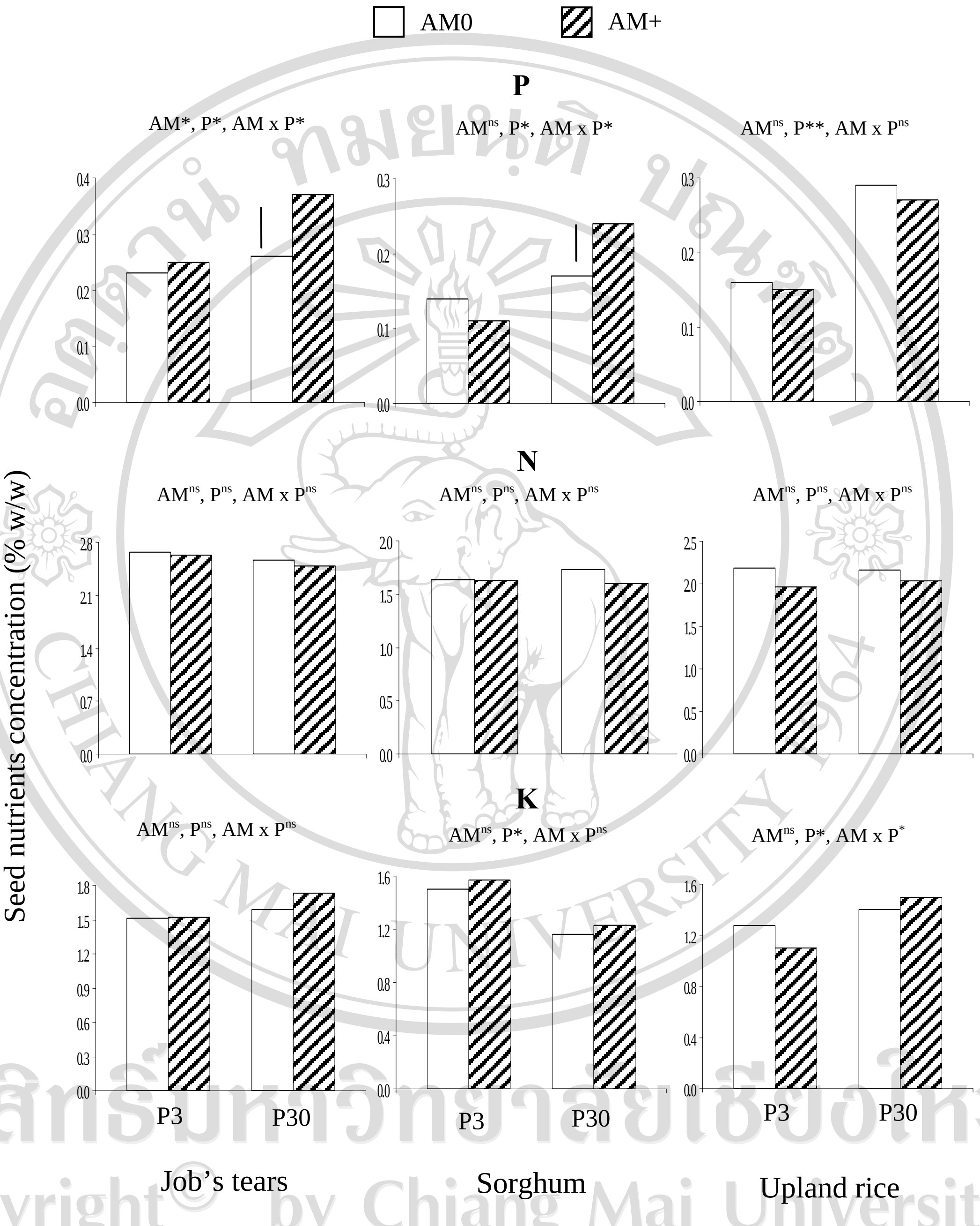


Figure 3.2 Whole seed nutrient concentration of Job's tears, sorghum and upland rice with two P levels (P3: 3 kg P ha^{-1}, P30: 30 kg P ha^{-1}) with and without AM inoculation. Vertical bar indicated significant differences of seed nutrient concentration by LSD $P < 0.05$.

3.3.5 Mycorrhizal responsiveness (MR) or mycorrhizal dependency (MD)

3.3.5.1 Percent of mycorrhizal growth responsiveness (%MGR)

There was an interaction ($P < 0.05$) between plant species and P application for mycorrhizal plant growth responsiveness (%MGR) at both harvests (Table 3.7). In the H1, percent mycorrhizal growth responsiveness at P3 (%MGR$_{P3}$) was highest in corn and Job's tear by 43, 49 %, respectively, followed by sorghum was 28% and upland rice had negative responded to inoculation was -12% (Table 3.7). By the H2, pada had highest responding to AM+ inoculation (99%), whereas %MGR$_{P3}$ did not differ in corn, Job's tears and sorghum. Upland rice had lowest %MGR$_{P3}$ (18%). Percent mycorrhizal growth responsiveness at P30 (%MGR$_{P30}$) decreased in all plant species, and only upland rice was the only crop species to show a negative response to inoculation at P30, it was -18% (Table 3.7).

3.3.5.2 Percent of mycorrhizal nitrogen responsiveness (%MNR)

Responses to AM+ inoculation was significant differrent between plant species and P application (Table 3.8). Mycorrhizal nitrogen responsiveness (%MNR) was decreased when increased P to P30 at both harvests. In the H1, the ranging of %MNR$_{P3}$ was 16 to 53% by the higest was Job's tears and the lowest was upland rice. By the H2, in AM0 pada at P3, there was not enough sample for analysis, and based on the dry weight produced only about 5-6 mg N pot^{-1} was estimated to have been taken up so %MGR$_{P3}$ was 97% whereas, in corn, Job's tears and sorghum were 40, 42 and 58%, respectively. Upland rice had lowest %MGR$_{P3}$ (23%). Mycorrhizal responsiveness was decreased by increasing P to P30 (Table 3.8).

3.3.5.3 Percent of mycorrhizal phosphate responsiveness (%MPR)

For mycorrhizal phosphate responsiveness (%MPR), there was a significant effect of P and plant species. At the H1, %MGR_{P3} were 55 – 63% in corn, Job's tears and sorghum and upland rice had lowest 21% (Table 3.9). By the H2, pada was highest %MPR_{P3} as 99% and followed by corn, Job's tears and sorghum were 51, 49 and 61% respectively, upland rice had lowest %MPR_{P3} as 35%. Mycorrhizal phosphate responsiveness in all plants was depressed by the addition of P30. The %MPR declined from 99 to 66% in pada and from 35 to 0% in upland rice for P3 and P30 plants, respectively (Table 3.9).

3.3.5.3 Percent of mycorrhizal potassium responsiveness (%MKR)

Plant species showed different response to AM+ inoculation for potassium uptake ($P < 0.05$). In the H1, Job's tears was a highest of %MKR_{P3} as 67%, followed by corn and sorghum were 38 and 34%, respectively. Upland rice had the lowest %MKR_{P3} as only 9%, however, at P30 it showed higher %MKR (28%) than at P3 (9%) whereas, %MKR of those species declined when were grown at P30. By the H2, pada had highest %MKR_{P3} as 99% and declined to 34% at P30. Job's tears showed responsiveness to AM+ inoculation at both P soils. Mycorrhizal potassium responsiveness (MKR_{P3}) was 31 and 69% in corn and Job's tears and decreased to -7 and -19%, respectively. Upland rice was the only one species that had a very low %MKR in both P soils (Table 3.10).

Table 3.7 Effects of phosphorus application and plant species on mycorrhizal growth responsiveness (MGR; %) for plant dry weight

Plant species	% MGR	
	3 kg P ha^{-1}	30 kg P ha^{-1}
	Harvest1	
Corn	42.8 A	18.4 CD
Job's tears	49.3 A	16.8 CD
Sorghum	27.8 B	8.2 DE
Upland rice	-12.1 E	5.8 DE
	Harvest2 (H2)	
Pada	99.4 A	48.3 BC
Corn	61.7 B	18.9 D
Job's tears	55.2 BC	35.3 CD
Sorghum	67.5 B	8.0 D
Upland rice	18.0 D	-18.7 E

Analysis of variance

Effect	F-test	
	H1	H2
S	**	**
P	**	**
S x P	**	*

Different letters in each harvest indicate significant differences between %MGR by LSD at $P < 0.05$ after arcsine transformation. * significant at $P < 0.05$, **$P < 0.01$.

Table 3.8 Effects of phosphorus application and plant species on mycorrhizal nitrogen responsiveness (MNR; %) at the two harvests

Plant species	% MNR	
	3 kg P ha^{-1}	30 kg P ha^{-1}
	Harvest1 (H1)	
Corn	44.6 AB	0.5 DE
Job's tears	52.9 A	10.0 D
Sorghum	31.8 BC	- 9.5 E
Upland rice	15.9 CD	2.1 DE
	Harvest2 (H2)	
Pada	97.0 A	19.9 CD
Corn	39.5 BC	9.7 DE
Job's tears	42.3 B	- 1.0 DE
Sorghum	58.5 B	- 25.0 E
Upland rice	23.0 CD	- 12.6 E

Analysis of variance

Effect	F-test	
	H1	H2
S	**	**
P	**	**
S x P	*	**

Different letters in each harvest indicate significant differences between %MNR by LSD at $P < 0.05$ after arcsine transformation. * significant at $P < 0.05$, **$P < 0.01$.

Table 3.9 Effects of phosphorus application and plant species on mycorrhizal phosphorus responsiveness (MPR; %) at the two harvests

Plant species	% MPR	
	3 kg P ha^{-1}	30 kg P ha^{-1}
	Harvest1 (H1)	
Corn	55.2 A	36.1 B
Job's tears	63.2 A	14.8 CD
Sorghum	54.8 A	2.8 D
Upland rice	20.7 BC	6.8 D
	Harvest2 (H2)	
Pada	98.9 A	66.5 B
Corn	51.2 B	13.6 D
Job's tears	49.1 B	27.2 CD
Sorghum	61.1 B	24.3 CD
Upland rice	34.6 C	0.0 D

Analysis of variance

Effect	F-test	
	H1	H2
S	**	**
P	**	**
S x P	*	*

Different letters in each harvest indicate significant differences between %MPR by LSD at $P < 0.05$ after arcsine transformation. * significant at $P < 0.05$, **$P < 0.01$.

Table 3.10 Effects of phosphorus application and plant species on mycorrhizal potassium responsiveness (MKR; %) at the two harvests

Plant species	% MKR	
	3 kg P ha^{-1}	30 kg P ha^{-1}
	Harvest1 (H1)	
Corn	38.0 B	13.2 DE
Job’s tears	67.0 A	1.7 E
Sorghum	34.3 B	1.7 E
Upland rice	9.4 DE	28.2 C
	Harvest2 (H2)	
Pada	99.6 A	34.0 DE
Corn	31.5 C	- 7.4 DE
Job’s tears	58.3 BC	23.2 CD
Sorghum	68.9 B	- 19.1 E
Upland rice	2.0 DE	- 2.2 DE

Analysis of variance

Effect	F-test	
	H1	H2
S	**	**
P	**	**
S x P	*	*

Different letters in each harvest indicate significant differences between %MKR by LSD at $P < 0.05$ after arcsine transformation. * significant at $P < 0.05$, **$P < 0.01$.

3.3.6 AM infection and spore density

No vesicles, arbuscules or associated spores were observed in the uninoculated control plants. Different degrees of AM root colonization were evident between the plant species at both harvests (Figure 3.3). In P3 plants at H1, corn already had the highest degree of AM colonization at 96%, followed by pada and Job's tears at about 80%, followed by sorghum at 74%, with the lowest degree of AM colonization of only 24% in upland rice. The plant species also differed in the degree that their AM root colonization was depressed at P30. The root colonization by AM was not significantly different between P3 and P30 in pada and upland rice. Increasing P from P3 to P30 depressed AM root colonization in sorghum by 23% and by 75% in corn and Job's tears (Figure 3.3). By H2 there were slight increases in root colonization in all species, but the differences between different crops remained about the same as in H1. The notable exception was corn, in which the effect of P was greatly lessened by H2. However, there was no correlation between root colonization and plant dry weight, yield or nutrient accumulation in any species. Less than 1 spore g^{-1} soil was found in AM treatments at the first harvest (data not shown). In P3 plants, by H2 Job's tears had the highest density of spores, at 40 spores g^{-1} soil, followed by pada, corn and upland rice with about half the number, and the lowest spores density at half these again in sorghum (Table 3.11). The number of spores were much lower at P30, where corn, Job's tears, pada and sorghum were all reduced to only 4-8 spores g^{-1} soil, while in upland rice the number was down to just 1 spore g^{-1} soil. Identified by morphology of their spores, *Acaulospora* was the most dominant genus of AM fungi. There were, however, some differences among the plant species and between P levels. *Acaulospora* represented almost all of the spores in the four food crops at P3 (Table

3.11) and a proportion of spore type at two P levels was shown in Figure 3.4. In pada at P3, *Acaulospora* and *Glomus* together equally represented 93% of the spores, with *Scutellospora* contributing 7%. When P was increased the dominance of *Acaulospora* declined in the food crops but increased in pada. At P30 *Acaulospora* became the dominant genus in pada representing almost 90% of the spores, and contribution of *Glomus* dropped from 47% in P3 to 7% in P30, whereas *Scutellospora* remained about the same. In contrast, in the four food crops, *Acaulospora* that accounted for virtually 100% of the spores in P3 had declined significantly in dominance in P30; to 77 % in jobs tears, 75% in sorghum, 65% in upland rice and 33% in corn. In sorghum and upland rice the share of spores was taken up by *Scutellospora*. In Job's tears 14% of the spores was taken up by *Glomus* and 9% by *Scutellospora*. In corn, *Scutellospora* took up 17% of the spores, while the remaining 50% were of spores of other genera, including *Archaeospora*, *Gigaspora* and *Paraglomus*. Spore samples were taken from rhizosphere soils of plants, those have shown in the Figure 3.5.

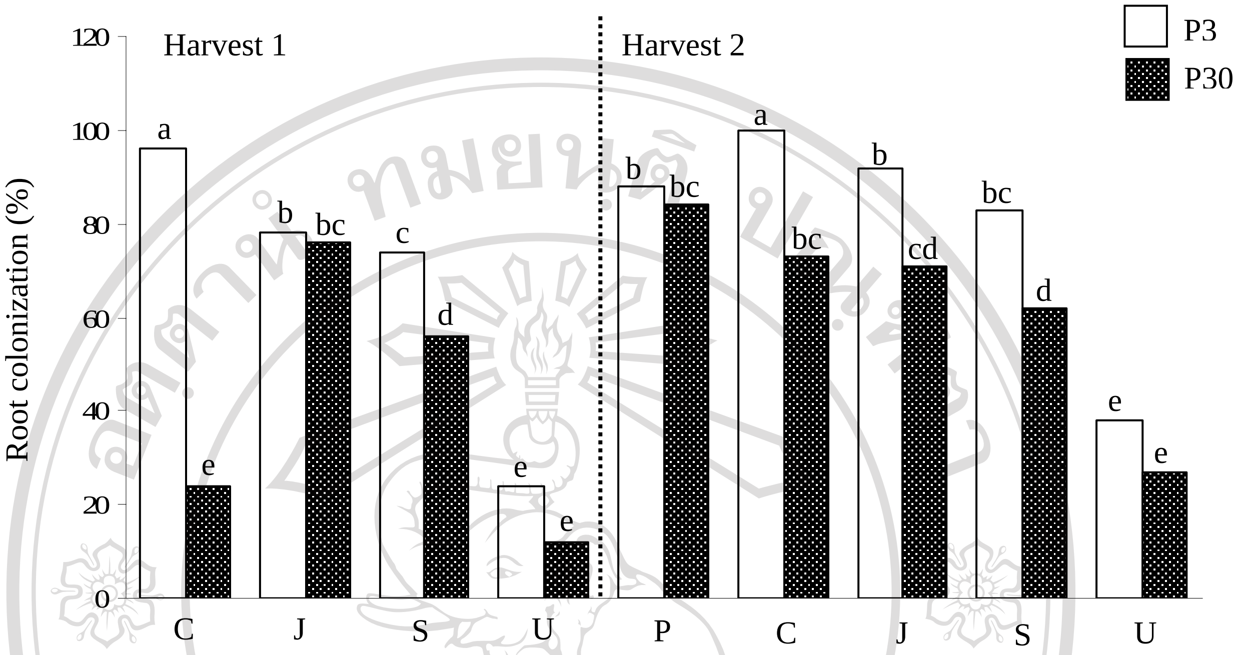


Figure 3.3 Root colonization of pada (P) at the one harvest and four swidden crops (C: corn, J: Job's tears, S: sorghum, U: upland rice) at two harvests as affected by AM inoculation at two P levels, 3 and 30 kg P ha^{-1}. Different letters above the columns indicate significant differences between root colonization within harvest by LSD at $P < 0.05$ after arcsine transformation.

Analysis of variance of root colonization (after arcsine transformation)

Harvest	Effects		
	S	P	S x P
1	**	**	**
2	**	**	**

* significant at $P < 0.05$, $^{**}P<0.01$

Table 3.11 Arbuscular mycorrhizal spores in pada and four swidden crops with two phosphorus levels

P level	Spore density (number g^{-1} soil				
	Pada	Corn	Job's tears	Sorghum	Upland rice
3 kg P ha^{-1}	21b	24b	40a	10c	19b
30 kg P ha^{-1}	8c	5d	4d	7cd	1e
% Spore decrease	62%	79%	90%	30%	95%
Analysis of variance					
Effect	S	P	S x P		
F-test	**	**	**		

Different letters indicate significant differences in total spore numbers g^{-1} soil by LSD at $P < 0.05$ after log transformation. ** significant at $P < 0.01$.

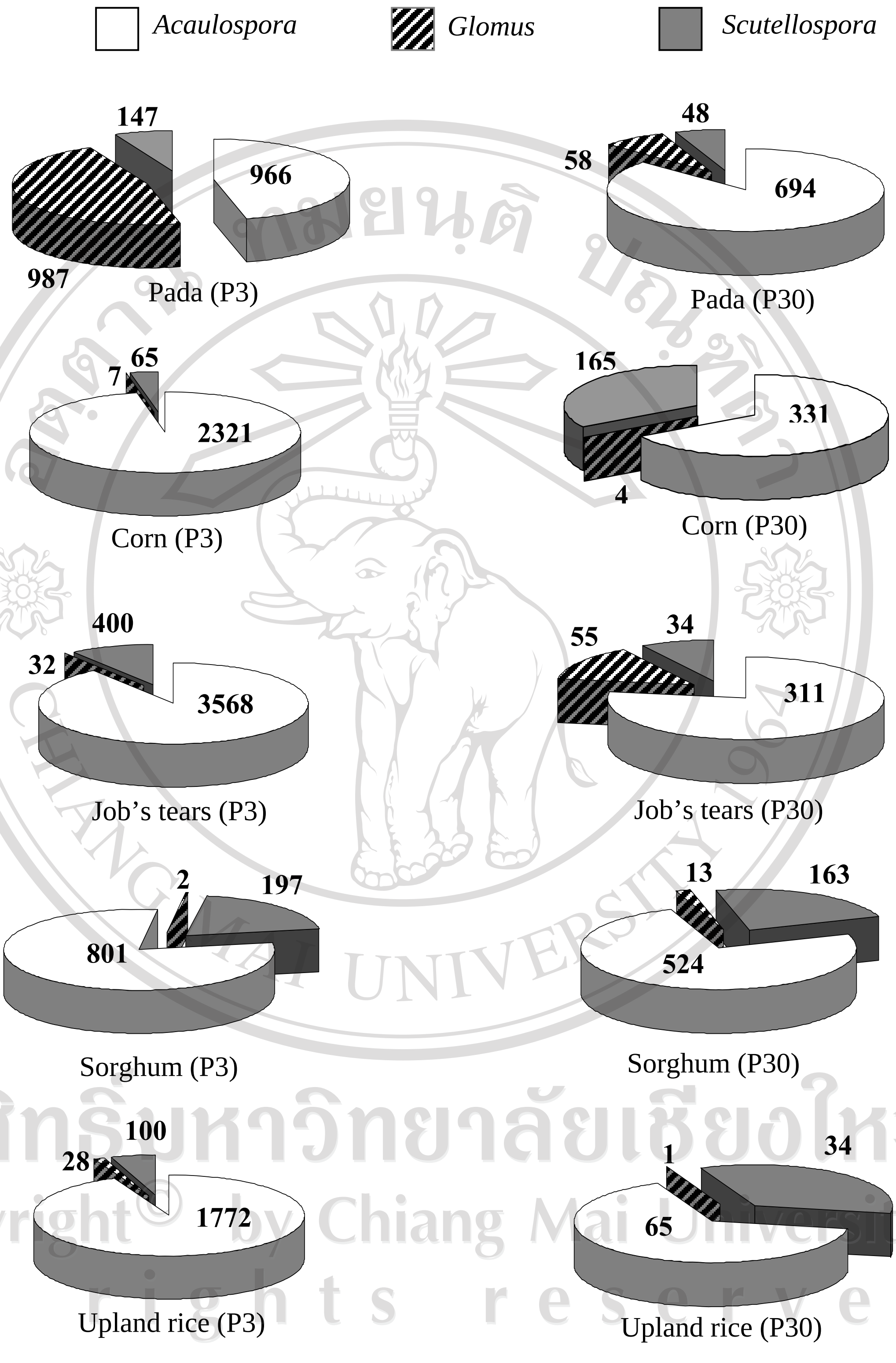
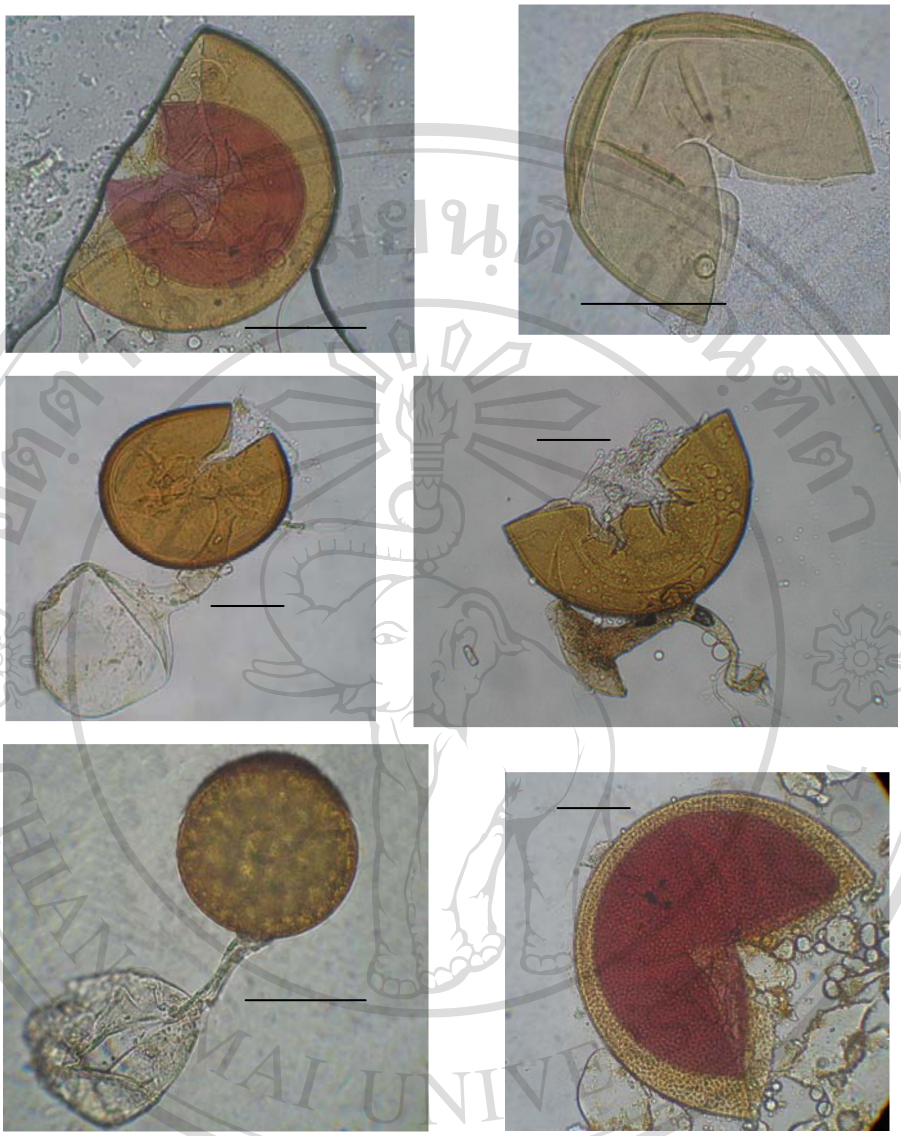
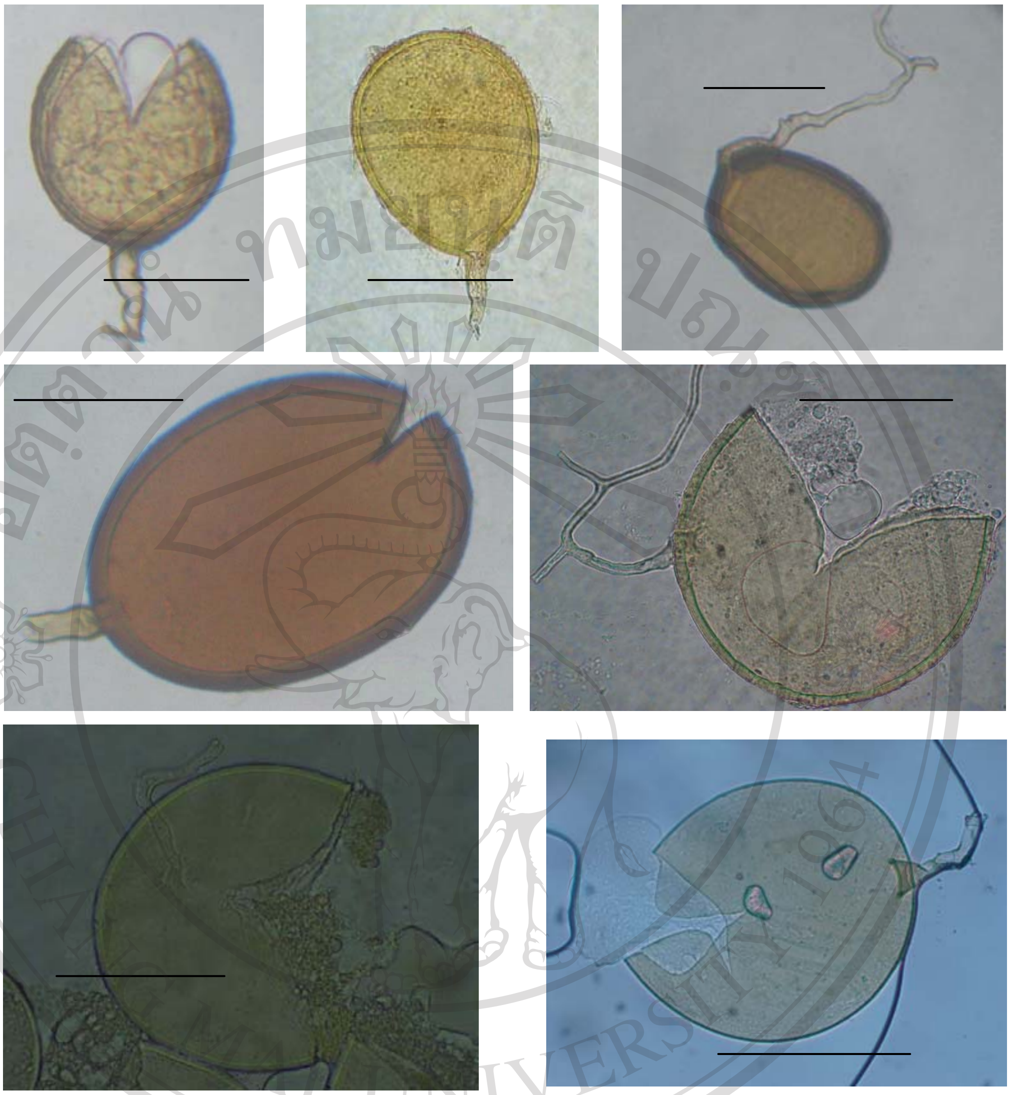


Figure 3.4 Effect of plant species on AM fungi spore type that developed from a soil inoculant of mixed spore types at two P levels. The pie sections indicate portion of the different genera, with number of spores of each genus 100 g^{-1} soil.

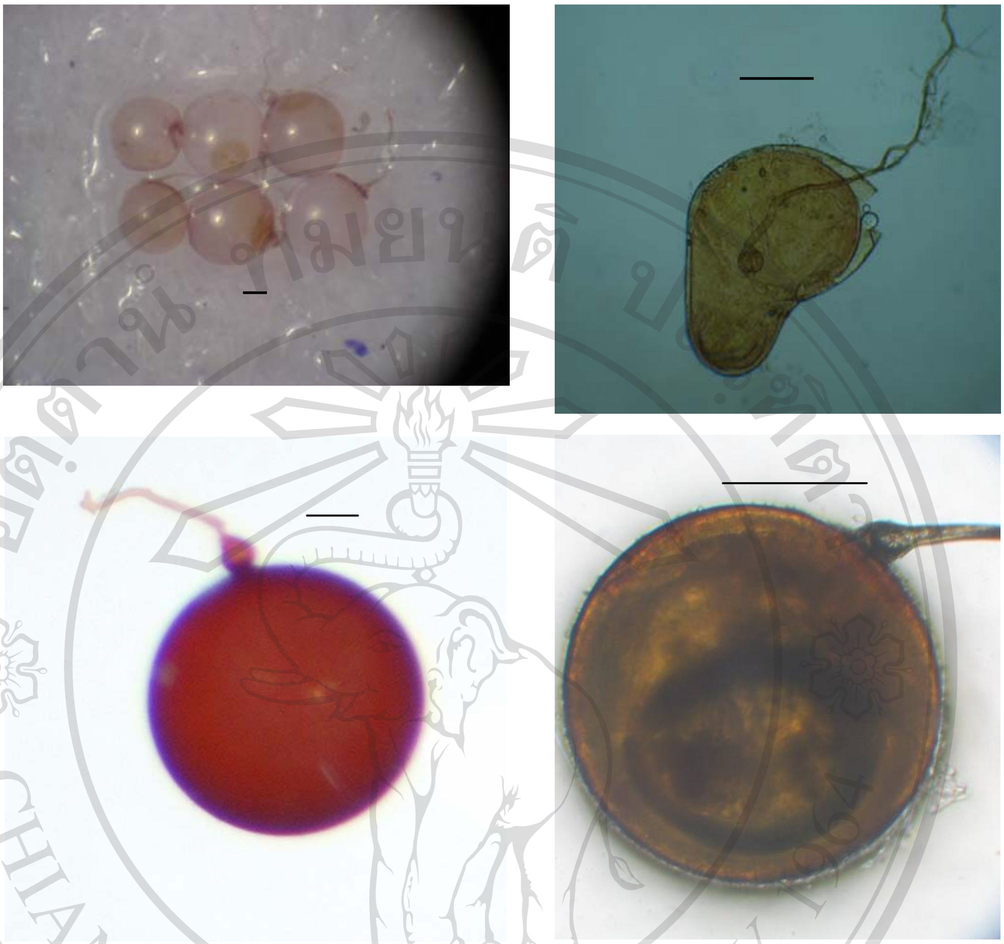


***Acaulospora* spp. (Bar = 50 µm)**

Figure 3.5 Spores of AM fungi from rhizosphere of host plants



***Glomus* spp. (Bar = 50 µm)**



***Scutellospora* spp. (a-c) and *Gigaspora* sp. (d)**

Bar = 50 µm

3.4 Discussion

Results from the pot experiment have shown that there was a wide range of responses in plant species tested to inoculation with soil containing AM fungal spores. In addition, the effect of AM fungi on total biomass of those responsive species was strongly dependent on the level of P application. AM inoculation increased total biomass of pada and most food crops at P3 but not in upland rice. The level of infection in the freely draining pots for upland rice was lowest when compared with other crops and pada and was similar to those recorded for paddy rice grown under flooded conditions and inoculated with AM fungi (20 to 39% infection, Secilia and Bagyaraj, 1992). Although, upland rice biomass did not respond to AM inoculation, grain yield of upland rice was increased by inoculation at P3.

Plants with an extensive (highly branched, long root hairs, numerous root hairs, thin roots) root surface have often been observed to derive little benefit from mycorrhizas (Koide *et al*., 1988; Hetrick, 1991). However, even though the root system of upland rice was highly fibrous with extensive root hairs, inoculation did increase the total N and P contents and moreover, the increase in seed yield from inoculation was through an increase in the percentage of filled grain and not in the number of spikelets when it was grown in the P-deficient soil (Table 3.3). It is suggested that the number of spikelets was determined before AM fungi had time to be effective in upland rice. Solaiman and Hirata (1997) found that wetland rice seedlings inoculated with AM fungi at the nursery stage under both dry and wet conditions increased growth, grain yield and nutrient acquisition under field conditions as well as in a pot experiment. Furthermore, aerobic rice inoculated with AM fungi produced more biomass than uninoculated rice (Gao *et al*., 2007).

In Job's tears, inoculation increased seed yield at both P3 and P30. Moreover, seed P concentrations were increased by inoculation and corresponding root infection levels were 92% and 71%, respectively. This plant is a tropical member of the family Poaceae (grass family). It is harvested as a cereal crop and is used for decoration, food, animal feed and medicine. It is also transferred outside the village for selling in the market. As well as in Laos, this crop is a cash crop conducting value for their farmers (NAFRI, 2005). The responsiveness of this crop to AM fungi is notable.

Corn and sorghum are cultured as host for multiplying spore and mycelium of AM fungi. The segments from these have used for studying effects of AM fungi on individual monoculture. The present study shows AM fungi improved these crops growth and nutrient uptake. Although, corn did not produce any ear but it was shown responses to AM fungi inoculation by increasing dry weight. Fertilizer application in the pots experiment may not sufficient for this plant until maturity. Although, AM fungi can increase plant production especially, in low P soil (P3) but it could not compensated of P acquiring as P30. No swidden crops showed high dependency to AM fungi like enriching tree pada (Table 3.8).

Rhizosphere in different food crops showed difference of spore number g^{-1} soil. In addition, the effect of P on spore number differed among plant species. The current experiment suggests that the host species may have influenced the AM fungal population as pada had equal spore densities of *Acaulospora* and *Glomus* whereas the rhizosphere of all food crops was dominated by *Acaulospora* and had few spores of *Glomus*. Phosphorus application to P30 also depressed total spore number g^{-1} soil in rhizosphere of the pada and crops but varied among the different species of plant (Table 3.7). Giovannetti *et al*. (1988) found that the ability of *Glomus monosporum* to produce spores and to enhance plant growth per unit infected root length depends on the host plant species. Further work is required to explore this aspect in more detail for the AM fungi at Huai Tee Cha village. *Acaulospora* was the highest genus at both P3 and P30 but species level was declined in P30. However, the proportion of *Scutellospora* genus did not depress by effect of P application while, a proportion of *Acaulospora* and *Glomus* genus were sensitively to P application. In a long-term field fertilizer trial with corn at Nakhon Sawan in central Thailand, that the population of AM fungi changed with soil fertility at a species level (Nabhadalung *et al.*, 2005). It was suggested that different genus of AM may be widely responding to soil fertilities (Figure 3.4). Although, *Acaulospora* was the dominant genus however, we do not know which genus/species was most effective for each food crop. It is not known whether the same species of AM fungi colonizing the roots at P3 were also active at P30. Therefore, single spore cultures need investigation and also important to investigate more than one mycorrhizal variable when study effects of AM fungi to host plant. High diversity of AM fungal spores in the rhizosphere of pada in the farmer’s fields should be advantageous for food crops especially if they differ in attributes that relate to their ability to access a range of soil P pools across fields.

In conclusion this study has shown how nutrient uptake of swidden crops in shifting cultivation can be greatly stimulated and seed yield boosted by association with AM fungi. It has also been shown that the AM effect differed considerably between crop species and was influenced by soil P.