CHAPTER 5

EFFECTS OF ARBUSCULAR MYCORRHIZAL FUNGI, SOIL ACIDITY AND PHOSPHORUS ON COWPEA

5.1 Introduction

The AMF from Huai Teecha village have been found to play the key role in soil fertility improvement, indirectly via a fallow enriching tree, *Macaranga denticulata* (Yimyam *et al.*, 2003) and directly on many crop species including upland rice, job's tears and sorghum (Wongmo, 2008). Evidence of close association between local legumes and AMF in acidic low P soil has been found in % root colonization, spore density and plant P status (chapter 3), but the benefit of the AMF to enhance legume growth in low P acid soil have never been tested. And if the legume can help local legumes to cope with acid soil problem in the upland shifting cultivation system, can they alleviate acid soil problem of legume in the other areas. The objective of this study is to evaluate effectiveness of AMF from Huai Teecha village to alleviate acid soil stress on cowpea.

5.2 Material and method

A pot experiment was conducted in a glasshouse at Chiang Mai University in the late cold season (26 January – 12 May 2007). The experiment was designed in a factorial of 3 factors in RCB with 4 replications. One pot was one experimental unit. The factors included 2 levels of soil pH (pH 5, acid soil and pH6.7, non-acid soil), 3 levels of phosphorus (P) application rate (16, 33 and 45 kg P/ha, designated P16, P33 and P45) and 2 levels AMF inoculation (inoculated, AM+ and uninoculated, AM0).

Plant growth medium was prepared from a mixture of sand and soil. Sansai soil (0-30 cm depth) was collected from Mae Hia agricultural research station and training center Chiang Mai University with the following properties: 3.8 mg Bray II P/kg and pH (1:1 H_2O) 5.9. The soil was air-dried, ground and then sieved to pass a 5 mm screen. The sieved soil was mixed with washed river sand in a 2:1 ratio (w/w). Then 3.6 kg growth medium was put in to plastic bag. The mix was adjusted pH to 5 (acid soil) or 6.7 (non acid soil) by adding $Al_2(SO_4)_3$ $18H_2O$ or $CaCO_3$, respectively. Basal nutrients were as follows (mg/kg): K_2SO_4=71, $CaCl_2.H_2O$=94, $MnSO_4.H_2O$=10, $ZnSO_4.7H_2O$=5, $CuSO_4.5H_2O$=2.1, H_3BO_3=0.8, $CoSO_4.7H_2O$=0.36 and $Na_2MoO_4.2H_2O$=0.18. The potting medium was autoclaved at 121°C for one hour. The seeds of cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Ratchathani, provided by Khon Kaen Field Crop Research Centre) were surface sterilized with 70% ethanol for 5 minutes then washed three times with sterilized water before sowing five seeds per pot. Each 5 L (314 cm^2 surface area) free-draining plastic pot contained 3.6 kg of potting medium. Each seed was inoculated with 1 ml of appropriate rhizobium suspension at 10^9 cells/ml (provided by Department of Soil Science Faculty of Agriculture Chiang Mai University). The P treatments were applied in form of KH_2PO_4. The AM+ treatment consisted of 50 g of soil inoculum, obtained from the root zone of *Mimosa invisa* growing for 8 months in soil from farmer's field from Huai Teecha, and AM0 treatment was the same weight of AM+ that had been autoclaved at 120°C for 1 hour. The inoculum in AM+ contained 2.9 mg P/kg at pH 5.5 and 25 AMF spores/g (66% *Glomus fulvum,* 17.7% *Acaulospora morrowiae,* 1% *Glomusclarum,* 1.4% *Glomus spp.*, 0.5% *Scutellospora erythropa* and 13% Unkown). At 10 days from sowing plants were thinned to 3 per pot. Plants were harvested at 50

days after sowing (at pod filling stage). Shoot were cut at ground level then oven dry at 75°C for 48 hours before weighed. Roots were washed free of soil. Root nodules were collected and oven dry before weighed. Fresh roots were weighed before cut around 1 cm long. Then a root sub-sample (10% of total root fresh weight) was randomly taken from every pot for AMF measurement. The remained root were oven dried before weighing. For AMF measurement root samples were cleared in 10% KOH before staining with 0.05% trypan blue in lactoglycerol. Root colonization percentage was assessed using the intercept method (Brundrett *et al.* 1996) under a compound microscope. Thirty-two pieces of root (one pieces was 1 cm long) were examined for each sample. Nitrogen concentration in root and shoot tissue were measured by Kjeldahl method. Shoot and root P concentration were measured by Molybdovanadate-Phosphoric Acid method (Murphy and Riley, 1962).

5.3 Results

Root colonization was not found in AM0 treatment. In AM+ treatment, root colonization ranged from 40 to 68%, but with no effect of soil pH and P level (Table 5.1).

Table 5.1 Root colonization in acid and non-acid soil varied with 3 different P levels.

	Applied P level		
Soil pH	P16	P33	P45
	Root colonization (%)		
pH 5 (Acid soil)	45.7	40.4	44.4
pH 6.7 (Neutral soil)	56.8	53.0	46.6
F-test	pH^{NS}	P^{NS}	$PxpH^{NS}$

Phosphorus level: P16 = 16 kg P/ha; P33 = 33 kg P/ha and P45 = 45 kg P/ha, NS = not significant

The effect of AMF on shoot dry weight of the cowpea highly depended on both soil acidity and P level (Table 5.2). In acid soil, AMF enhanced shoot growth in every P level, with the biggest effect of AMF at P33. In acid soil the AMF inoculation increased shoot dry weight by 32, 73 and 21 % in P16, P33 and P45 respectively. In non acid soil the response to AMF was less and detectable only at P16 where shoot dry weight was increased 29% (Table 5.2).

Table 5.2 Effect of AMF and P application on cowpea shoot dry weight (g/pot) in acid and non-acid soil

	Acid soil				Non-acid soil		
Applied P	AM0		AM+		AM0		AM+
P16	2.10 h		2.78 fg		2.42 gh		3.12 f
P33	2.85 fg		4.94 cd		4.30 de		4.93 cd
P45	4.21 e		5.10 bc		6.18 a		5.74 ab
F-test	AM**	pH**	P**	AMxpH**	AMxP**	pHxP*	AMxpHxP*
$LSD_{0.05}$	0.27	0.27	0.33	0.38	0.46	0.46	0.65

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$

There was no three factors interaction on root dry weight and total dry weight but there was significant interactions of AM x P, AM x pH and pH x P (Table 5.3 and 4.4). For the interaction between P and soil pH, P application increased root and total weight in both soil pH but the response was slightly less in acid soil. Without AMF soil acidity depressed root and total weight for 25% and 28% respectively but the root and total weight of inoculated plant was not affected by soil acidity (Table 5.3 and 4.4). For the interaction between P and AMF, AMF increased root and total weight in P16 and P33 but had no effect in P45 (Table 5.3 and 5.4).

Table 5.3 Effect of AMF and P application on cowpea root dry weight (g/pot) in acid and non-acid soil

	Acid soil		Non-acid soil		Mean	
Applied P	AM0	AM+	AM0	AM+	AM0	AM+
P16	1.18	1.75	1.34	1.68	1.26 c	1.72 b
P33	1.48	2.46	2.13	2.33	1.81 b	2.39 a
P45	2.10	2.38	2.88	2.67	2.49 a	2.52 a
Mean	1.58 B	2.20 A	2.12 A	2.23 A	1.85	2.21

F-test	AM**	pH**	P**	AMxpH**	AMxP**	pHxP**	AMxpHxP^{NS}
$LSD_{0.05}$	0.13	0.13	0.16	0.18	0.22	0.22	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$ the upper case for comparing the interaction of AMxpH and the lower case for the interaction of AMxP

Table 5.4 Effect of AMF and P application on cowpea total dry weight (g/pot) in acid and non-acid soil

	Acid soil		Non-acid soil.		Mean	
Applied P	AM0	AM+	AM0	AM+	AM0	AM+
P16	3.17	4.50	3.75	4.80	3.46 d	4.65 c
P33	4.35	7.40	6.40	7.28	5.38 b	7.34 a
P45	6.30	7.50	9.17	8.40	7.73 a	7.95 a
Mean	1.58 B	2.20 A	2.12 A	2.23 A	5.52	6.65

F-test	AM**	pH**	P**	AMxpH**	AMxP**	pHxP*	AMxpHxP^{NS}
$LSD_{0.05}$	0.41	0.41	0.51	0.59	0.72	0.72	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$ the upper case for comparing the interaction of AMxpH and the lower case for the interaction of AMxP

The response of nodule dry weight to AMF depended on soil pH (Table 5.5). Although AMF increased nodule dry weight in both soil pH but the response was bigger in acid soil. In acid soil AMF inoculation increased nodule dry weight for 135% while nodule dry weight was increased just 48% in non-acid soil (Table 5.5).

Table 5.5 Effect of AMF and P application on cowpea nodule dry weight (mg/pot) in acid and non-acid soil

	Acid soil		Non-acid soil		P Mean
Applied P	AM0	AM+	AM0	AM+	
P16	10	96	23	102	58 c
P33	74	238	132	244	172 b
P45	184	297	257	264	251 a
Mean	89 C	210 A	137 B	203 A	

F-test	AM**	pH	P**	AMxpH*	AMxP	pHxP	AMxpHxP
$LSD_{0.05}$	26	-	32	37	-	-	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$ the upper case for comparing in the same row and the lower case for the same column

For nutrient status in plant, there were not any interaction between AMF, soil pH and P level but the effect of every single factor was found. Soil acidity depressed shoot P concentration in every P level and in both AM0 and AM+ (Table 5.6). Arbuscular mycorrhizal fungi inoculation increased shoot P concentration in every P level and in both soil pH (Table 5.6). Increasing P level increased shoot P concentration.

Table 5.6 Effect of AMF and P application on shoot P concentration (% w/w) in acid and non-acid soil

Applied P	Acid soil		Non-acid soil		P Mean
	AM0	AM+	AM0	AM+	
P16	0.097	0.121	0.094	0.125	0.109 c
P33	0.108	0.129	0.121	0.141	0.125 b
P45	0.124	0.141	0.139	0.147	0.138 a
Mean	0.120 B		0.128 A		
	AM0		AM+		
Mean	0.114 B		0.134 A		
F-test	AM**	pH**	P**	AMxpH^{NS}	AMxP^{NS}	AMxpHxP^{NS}
$LSD_{0.05}$	0.006	0.006	0.007	-	-	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$ the upper case for comparing in the same row and the lower case for the same column

Although there was no 3 factors interaction on total P content, interactions were found between AMF and P level and soil pH and P application (Table 5.7). Plant P content increased linearly with applied P, but with much stronger response in acidic than in non-acidic soil. AMF increased P content in all P level but the biggest effect was found in lowest P level (P16). The effect of the fungi diminished with increasing level of P application.

Table 5.7 Effect of AMF and P application on total P content (mg/pot) of cowpea in acid and non-acid soil.

Applied P	Acid soil			Non-acid soil			Mean	
	AM0	AM+	Mean	AM0	AM+	Mean	AM0	AM+
P16	3.29	6.25	4.77	3.70	7.18	5.44	3.49 e	6.72 d
P33	4.88	10.49	7.69	7.76	12.58	10.17	6.32 d	11.54 b
P45	8.00	11.78	9.89	12.06	13.79	12.93	10.03 c	12.78 a
F-test	AM**	pH**	P**	AMxpH^{NS}	AMxP**	pHxP**	AMxpHxP^{NS}	
$LSD_{0.05}$	0.51	0.51	0.63	-	0.89	0.89	-	

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$

Phosphorus uptake per unit root weight was used as an indicator of P uptake efficiency. Arbuscular mycorrhiza increased P uptake efficiency in both soil pH and in every P level (Table 5.8).

Table 5.8 Effect of AMF and P application on P uptake per unit root weight (mg P/g root) in acid and non-acid soil.

Applied P	Acid soil		Non-acid soil		Mean
	AM0	AM+	AM0	AM+	
P16	2.76	3.58	2.77	4.31	3.36 c
P33	3.28	4.25	3.63	5.20	4.09 b
P45	3.80	4.97	4.11	5.21	4.52 a
Mean	3.77 B		4.20 A		
	AM0		AM+		
Mean	3.39 B		4.59 A		

F-test	AM**	pH**	P**	AMxpH^{NS}	AMxP^{NS}	pHxP^{NS}	AMxpHxP^{NS}
$LSD_{0.05}$	0.24	0.24	0.29	-	-	-	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$ the upper case for comparing in the same row and the lower case for the same column

The 3 factors interaction was not found on shoot N concentration but interaction between P level and AMF was significant. In AM0 P application depressed shoot N concentration but in AM+ shoot N was stable along P levels (Table 5.9).

Table 5.9 Effect of AMF and P application on shoot N concentration (% w/w) in acid and non-acid soil

	Acid soil		Non-acid soil		Mean	
Applied P	AM0	AM+	AM0	AM+	AM0	AM+
P16	3.25	2.44	3.15	2.57	3.20 a	2.51 bc
P33	2.57	2.60	2.31	2.67	2.44 bc	2.64 bc
P45	2.32	2.61	2.43	2.86	2.37 c	2.74 b
F-test	AM^{NS}	pH^{NS}	P**	$AMxpH^{NS}$	AMxP**	$pHxP^{NS}$	$AMxpHxP^{NS}$
$LSD_{0.05}$	-	-	0.22	-	0.31	-	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at $P < 0.01$, means followed by different letter are significant different at $P < 0.05$

The interaction of AMxpH and AMxP on total N content was significant (Table 5.10). AMF increased total N content in acid soil but it had no effect in non-acid soil. The AMF inoculation increased total N in plant in P33 and P45 for 47 and 13% respectively but had no effect in P16 (Table 5.10).

Table 5.10 Effect of AMF and P application on total N content (mg/pot) in acid and non-acid soil

Applied	Acid soil		Non-acid soil		Mean	
P	AM0	AM+	AM0	AM+	AM0	AM+
P16	94.8	101.0	104.4	114.3	99.6 d	107.7 cd
P33	102.3	176.5	137.4	176.9	119.8 c	176.7 ab
P45	134.3	179.5	201.6	199.5	168.0 b	189.5 a
Mean	110.5 B	152.3 A	147.8 A	163.6 A	129.1	158
F-test	AM**	pH**	P**	AMxpH*	AMxP**	pHxP	AMxpHxP^{NS}
LSD0.05	10.4	10.4	12.8	14.7	18.0	-	-

AM = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, * = significant at $P < 0.05$, ** = significant at $p<0.01$, ** = significant at $p<0.01$, means followed by different letter are significant different at $p<0.05$ the upper case for comparing the interaction of AMxpH and the lower case for the interaction of AMxP

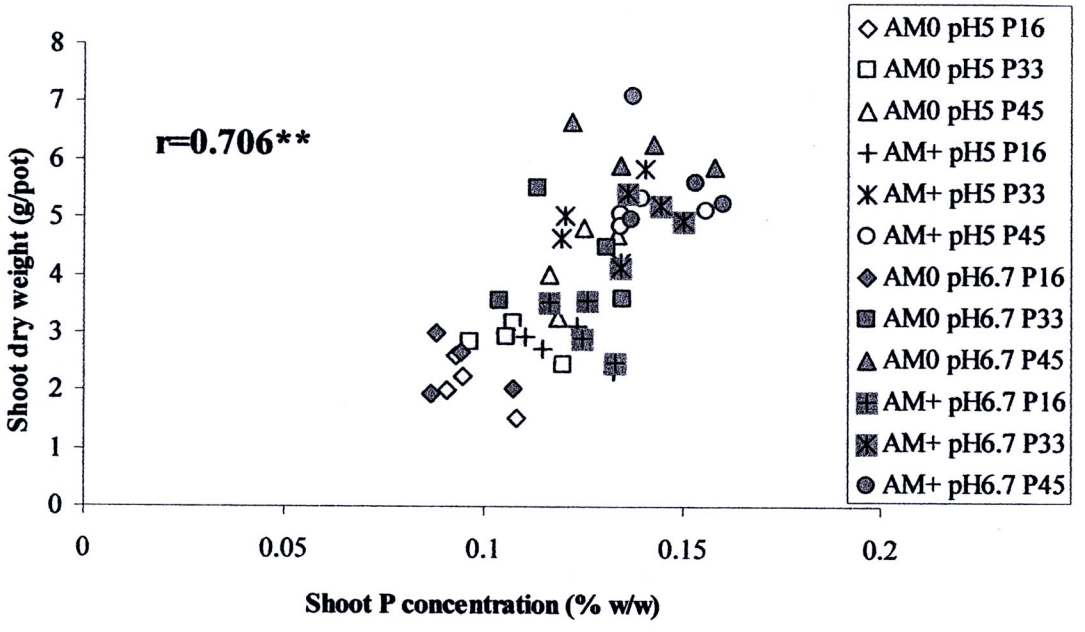

Figure 5.1 Correlation between shoot P concentration and shoot dry weight. r = correlation coefficient, ** = significant at $P < 0.01$

5.4 Discussion

In chapter 3 (survey work) root colonization highly depended on soil pH and soil P (Table 3.1) but in this experiment no effect of soil pH and soil P was found on root colonization. The P levels in this experiment were chosen from chapter 4 experiment 4.2.3. These P levels provided 25 to 66% of maximum yield that was suggested as suitable P level for mycorrhiza (Brundrett *et al.*, 1996), with just 2 soil pH level (5 and 6.7). Range of the P level and soil pH may not have been wide enough to see the effect on root colonization. Nevertheless growth response to AMF was highly dependent on P level and soil pH (Table 5.2 to 4.5). These results indicate (a) that response of plant to AMF was more sensitive to soil P and soil pH than root colonization, and (b) root colonization may or may not be related to plant response. Shoot P concentration (refer to P status in plant) strongly correlated with shoot growth

(Figure 5.1). This indicated that P deficiency was the limiting factor of cowpea growth. Soil acidity depressed cowpea growth by accentuating P deficiency. P status in cowpea was lowed by soil acidity (Table 5.6). Therefore correcting soil acidity problem is alleviating P deficiency problem. The AMF made cowpea more tolerant to soil acidity (Table 5.2 to 4.5) because AMF improved P status in cowpea (Table 5.6 and Figure 5.1). The inoculated plant had higher P status than un-inoculated control because it uptake P more efficient than control showing by higher P uptake per unit root weight of inoculated cowpea (Table 5.8). Many authors mention the advantage point of colonized plant as more nutrient uptake area. External mycelium of AMF explore to soil beyond host plant root and distinctly enlarges uptake surface area. Especially P is an un-mobile nutrient in soil. Enlarging uptake surface of AMF significantly enhance P uptake (Hayman, 1986; Marchner, 1995; Dell, 2002). The benefit of AMF is highly depended on soil P and soil pH. Cowpea got more benefit from AMF when it is grown in acid soil (Table 5.3 to 4.5) because soil acidity accentuated P deficiency as described above. But very low soil P (in P16) diminished benefit of AMF (Table 5.2 to 5.4) because P competition between host plant and AMF (Bethlenfalvay, 1992). High N accumulation in plant tissue was found in AM0 acid soil that plant had smallest growth and was in the extremely stress condition of P deficiency (Table 5.9 and 5.2). When plant was applied with P or inoculated with AMF the N concentration in tissue was diminished (Table 5.9) by higher plant growth. This result indicated that N deficiency was not the limiting factor in this experiment and suggested that plant growth is more sensitive to available P supply than nitrogen fixation.

Soil acidity depressed cowpea growth by accentuating P deficiency. The AMF from Huai Teecha village were effective in alleviating acid soil stress in cowpea because they improve P status in plant by improving P acquisition of host plant.