CHAPTER 4

EVALUATING SYSTEM FOR TESTING EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON LEGUME GROWTH IN ACID SOIL

4.1 Introduction

From the survey work in chapter 3 soil acidity and low soil P stimulated greater association between arbuscular mycorrhizal fungi (AMF) and legumes in Huai Teecha village. This result leads to a hypothesis that AMF help legumes to overcome acid soil constraints. To prove this, pot experiments need to be conducted. However, since in Chapter 3 root colonization was found consistently to be inversely associated with soil P, it is necessary to first establish the level of P where the benefit from AMF is maximized. Although benefits to plant growth from association with AMF are well known (Dell, 2002), responses to AMF in plants can also vary from positive, to no response and even to negative. In soils where supply of available P is not limiting the benefit from AMF is normally not realized (Plenchette and Morel, 1996). Negative effects of AMF on the host plant have been reported both when soil P is too high (Peng *et al.*, 1993) as well as too low (Janos, 2007). At extremely low available soil P AMF can depress host plant growth because the fungi compete with the host plant for a limited resource. In order to evaluate effectiveness of AMF, it is therefore necessary to identify the level of soil P where the benefit from AMF is maximized. It has been suggested that benefit from AMF is maximized at the soil P level that support 60% of maximum growth (Brundrett *et al.*, 1996). Moreover as P availability is influenced by soil acidity (Haynes and Ludecke, 1981), there is a possibility of an interaction between the effect of P and soil acidity on AMF response. The objective

of this set of studies is to find the suitable P level for testing benefit of AMF on legumes in acid soil.

4.2 Materials and methods

This study consisted of 3 pot experiments.

4.2.1 Comparing the effect of abuscular mycorrhizal fungi from Huai Teecha village on growth of cowpea in low P acid and nonacid Soil

The experiment was conducted in January 2006 in a greenhouse of Agronomy Department Faculty of Agriculture Chiang Mai University. Plant growth medium was prepared from a mixture of sand and soil. The soil used for the mixture was Sansai soil collected from Mae Hia Agricultural Research Station and Training Center, Chiang Mai University. It contains 4.1 mg P/kg (Bray II method) with pH 5.7 (1:1 H_2O). The soil was air-dried before grinding and sieved through 5 mm mesh screen and then mixed thoroughly with washed river sand in a 2:1 ratio (w/w). The growth medium pH was adjusted to 4.5, 5, 5.5 and 6.5 by adding $Al_2(SO_4)_3$ $18H_2O$ or $CaCO_3$. The prepared growth medium was autoclaved at 121°C for one hour twice before being used.

The experiment was designed as split plot with 6 replications. The main plots were inoculation with AMF (AM+) and no inoculation (AM0). The AM+ treatment consisted of spores extracted from 30 g of soil (pH 4.9 available P 3.4 mg/kg (Bray II method)) from Huai Teecha village Sop Moei district Mae Hong Son province using wet sieving and sucrose centrifugation methods. The fungi inoculums with 230 spores per pot from Huai Teecha soil were placed on a filter paper under the seeds when

sowing. Sub plots were 4 pH levels; 4.5, 5, 5.5 and 6.5. The plant used was cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee), supplied by Khon Kaen Field Crop Research Center. Seeds were surface sterilized with 70% ethanol for 5 min then washed three times with sterilized water before growing plants. Five surface sterile seeds were grown in 5 liters of drained plastic pots containing 4.5 kg of the growth medium. Each seed was inoculated with 1 ml of *Bradyrhizobium sp.* suspension (10^9 cells/ml) when sowing. Seedlings were thinned to remain 4 plants per pot at one week after emergence. Plants were harvested at 25 days after emergence. Shoots were cut at ground level. Roots were carefully washed free of soil. Nodules were counted and collected from the fresh root. Ten percent by weight of fresh root was sampled from every pot to measure mycorrhizal root colonization. For AMF measurement root samples were clear by soaking in 10% KOH for a day then stained with 0.05% trypanblue in lactoglyceral for a day. 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. The remained root and shoot and nodule were oven dry before weighed.

4.2.2 Testing effectiveness of commercial AMF inoculum to enhance cowpea growth in acid soil

The experiment was conducted from May to June 2006 in the same glass house as the experiment 4.2.1. The mixture of soil and sand (2:1 ratio w/w) as above was used as a growth medium. The soil used for the mixture was collected and prepared same as previous experiment. The soil had pH 5.76 and contained 2.72 mg P/kg soil. The growth medium pH was adjusted to 5.25 and 6.7 by adding $Al_2(SO_4)_3$

$18H_2O$ or $CaCO_3$. The growth medium was fertilized with (mg/kg soil) KH_2PO_4 (36), K_2SO_4 (71), $CaCl_2H2O$ (94), Mn SO_4H_2O (10), $ZnSO_47H_2O$ (5), $CuSO_45H_2O$ (2.1), H_3BO_3 (0.8), $CoSO_47H_2O$ (0.36), $Na_2MoO_42H_2O$ (0.18). The prepared growth medium was autoclaved at 121°C for an hour twice in consecutive day before being used. The experiment was arranged as factorial 2 factors with four replications under glass house condition. The factors were included 2 AMF treatments with (AM+) and without (AM0) inoculation and 2 soil pH acid (pH 5) and non acid (pH 6.7) soil. The plant used was cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee), supplied by Khon Kaen Field Crop Research Center. Cowpea seeds were surface sterile by soaking with 70% ethanol for 5 minute and washed three times with sterilized water before sowing. Five surface sterile seeds were grown in 5 liters of drained plastic pots containing 3.6 kg of growth medium. In Rh+ treatment each seed was inoculated with 1 ml of *Bradyrhizobium sp.* suspension (10^9 cells/ml) at sowing. In AM+ treatment, seeds were covered by 30 g of commercial AM inoculum (Mycorstar: P 16.8 mg/kg, pH 6.3) containing 30 AM spores /g. The autoclaved incoulum was used in control treatment (AM0). One week after emergence seedlings were thinned to remain 3 plants /pot. At 23 days after sowing every pot was fertilized 73 kg P/ha 228 mg P/pot in from of KH_2PO_4. At 30 days after sowing, plants were spayed with 0.05% H_3BO_3 solution. Plants were harvested at 45 day after sowing (pod filling stage). Shoots were cut at ground level. Roots were washed free of soil. Root nodules were counted and collected. The roots were weighed and sampled for mycorrhizal measurement as described in experiment 4.2.2. The remained root, shoot and nodule were oven dry before weighed. Nitrogen concentration in shoot and root

were measured by Kjeldahl method. Shoot and root P concentration were measured by Molybdovanado-Phosphoric Acid method (Murphy and Riley, 1962).

4.2.3 Comparing effect of soil acidity on cowpea growth at varying soil P level

The pot experiment was conducted in the same glass house as previous experiment from 21st September to 3rd November 2006. Plant growth medium was prepared from the mixture of sand and soil. The soil was Sansai soil and collected from Mae Hia experiment station. The soil cotained 3.5 mg P/kg and had pH 5.9. The soil was air dry be fore grinded and sieved past 5 mm screen then completely mixed with washed river sand in a 2:1 ratio (w/w). The growth medium pH was adjusted to 5.5 and 6.7 by adding $Al_2(SO_4)_3$ $18H_2O$ or $CaCO_3$ respectively. The growth medium was applied with fertilizer as following (mg/kg) K_2SO_4 = 71, $CaCl_2H_2O$ = 94, Mn SO_4H_2O = 10, $ZnSO_47H_2O$ = 5, $CuSO_45H_2O$ = 2.1, H_3BO_3 = 0.8, $CoSO_47H_2O$ = 0.36, $Na_2MoO_42H_2O$ = 0.18. The prepared growth medium was autoclaved at 121°C for one hour. The plant used is cowpea (*Vigna unguiculata* L. Walp.cv. Ubon Rajathanee), supplied by Khon Kaen Field Crop Research Centre. Seeds were surface sterilized by soaking in 70% ethanol for 5 minutes then washed three times with sterilized water before growing plants. Five surface sterilized seeds were grown in 5 L of drained plastic pot containing 3.6 kg of growth medium (314 cm^3 surface area in each pot). The pots were applied with 9, 16, 33, 45, or 82 kg P/ha (P9, P16, P33, P45 and P82 respectively) in form of KH_2PO_4. Every seed was inoculated with 1 ml of *Bradyrhizobium sp.* suspension (10^9cell/ml). At ten days after emergence plants were thinned to remain 3 plants /pot.

The experiment was arranged in Factorial 2 factors in Complete Randomize Design with 4 replications. The first factor was soil pH and the second factor was P application rate. A pot is an experimental unit. At 43 day after sowing plants were harvested. Shoots were cut at soil surface. Roots were carefully washed to free from soil. Root nodules were counted and collected. Root fresh weight was record before taken sub sample for AMF measurement as described in experiment 4.2.1. The remained root and shoot and nodule were oven dry for 48 hours before weighed. Nitrogen concentration in shoot and root were measured by Kiedahl method. Shoot and root P concentration were measured by Molybdovanado-Phosphoric Acid method (Murphy and Riley, 1962). Two soil cores (0.5 inches diameter, 10 cm long) were taken from every pot for soil P analysis. Soil P was measured by Bray II method.

4.3 Result

4.3.1 Comparing the effect of abuscular mycorrhizal fungi from Huai Teecha village on growth of gowpea in low P acid and non-acid soil

No root colonization was found in AM0 treatment. In AM+ treatment root colonization was very low (ranging from 0.3 to 4.8%) and not affected by soil pH (Table 4.1). Root dry weight, total dry weight and nodule number were not affected by soil acidity or AM treatment (Table 4.3, 4.4 and 4.6). But above ground biomass was depressed by soil acidity when cowpea was grown in soil pH 4.6 (Table 4.2) while nodule dry weight was depressed at soil pH 4.9 or below (Table 4.5).

Table 4.1 Effect of soil pH on mycorrhiza root colonization in cowpea at 25 days after sowing. (No root colonization was found in AM0)

Soil pH	AMF root colonization (%)
4.6	3.0
4.9	0.3
5.4	4.8
6.2	0.8
F-test	NS

NS = nonsignificant at $P < 0.05$

Table 4.2 Effect of soil pH and arbuscular mycorrhizal fungi inoculation on shoot dry weight (g/pot) of cowpea at 25 days after emergence

	Soil pH				Mean
	4.6	4.9	5.4	6.2	
AM0	1.201	1.482	1.547	1.674	1.476 B
AM+	1.124	1.226	1.372	1.408	1.283 A
mean	1.162 a	1.354 ab	1.459 b	1.541 b	
F-test	AM*	pH*	AMxpH^{NS}		

AM = arbuscular mycorrhizal fungi inoculation, pH=soil pH, * = singnificant different at $P < 0.05$, NS = non-significant, means followed by different letter are significant different at $P < 0.05$, the uppercase for comparing in the same column and the lower case for comparing in the same row

Table 4.3 Effect of soil pH and mycorrhiza inoculation on root dry weight (g/pot) of cowpea at 25 days after emergence

	Soil pH				mean
	4.6	4.9	5.4	6.2	
AM0	0.537	0.572	0.645	0.625	0.595
AM+	0.647	0.639	0.512	0.599	0.599
mean	0.592	0.606	0.579	0.612	
F-test	AM^{NS}	pH^{NS}	$AMxpH^{NS}$		

AM = arbuscular mycorrhiza fungi, pH = soil pH, NS = non-significant

Table 4.4 Effect of soil pH and mycorrhiza inoculation on total dry weight (g/pot) of cowpea at 25 days after sowing

	Soil pH				Mean
	4.6	4.9	5.4	6.2	
AM0	1.74	2.05	2.19	2.30	2.07
AM+	1.77	1.87	1.88	2.01	1.88
mean	1.75	1.96	2.04	2.15	
F-test	AM^{NS}	pH^{NS}	$AMxpH^{NS}$		

AM = arbuscular mycorrhiza fungi, pH = soil pH, NS = non-significant

Table 4.5 Effect of soil pH and mycorrhiza inoculation on nodule dry weight (mg/pot) of cowpea at 25 days after emergence

	Soil pH				mean
	4.6	4.9	5.4	6.2	
AM0	15	15	27	29	21 A
AM+	18	27	33	42	30 B
mean	16 a	21 ab	30 bc	35 c	
F-test	AM*	pH**	AMxpH^{NS}		

AM = arbuscular mycorrhiza fungi, pH = soil pH, * = singnificant different at $P < 0.05$, ** = significant at $P < 0.01$, NS = non-significant, means followed by different letter are significant different at $P < 0.05$, the uppercase for comparing in the same column and the lower case for comparing in the same row

Table 4.6 Effect of soil pH and mycorrhiza inoculation on nodule number/pot of cowpea at 25 days after emergence

	Soil pH				mean
	4.6	4.9	5.4	6.2	
AM0	174	184	269	276	226
AM+	177	207	174	199	189
mean	175	196	222	237	
F-test	AM^{NS}	pH^{NS}	AMxpH^{NS}		

AM = arbuscular mycorrhiza fungi, pH = soil pH, NS = non-significant

4.3.2 Testing effectiveness of commercial AMF inoculum on cowpea growth in acid soil

The contamination was found in AM0 control treatment. Two and six percent of root colonization was found in acid and non-acid soil respectively. Inoculating with commercial inoculum increased root colonization to 99% in acid and 100% in non-acid soil. Soil acidity had no effect on root colonization (Table 4.7). Biomass yield was not affected by AMF. Soil acidity depressed shoot, root and total dry weight for 28.6, 27.6 and 28.6 % respectively (Table 4.7). Soil acidity and AMF had no effect on nodule dry weight (Table 4.7) but nodule number was 27.7% depressed by AMF (Table 4.8). Shoot P and N concentration were not affected by soil acidity or AMF but total P and N in plant were 25.7 and 22.3% depressed by soil acidity (Table 4.8).

Table 4.7 Effect of AMF and soil acidity on root colonization, shoot, root, nodule and total weight and nodule number of cowpea applied with N fertilizer or inoculated with rhizobium

	Root colonization (%) #	Shoot weight (g/pot)	Root weight (g/pot)	Total weight (g/pot)	Nodule weight (g/pot)
			pH 5		
AM0	2	11.3	1.9	13.2	0.677
AM+	99	10.6	2.2	12.8	0.711
mean	51	11.0	2.1	13.0	0.694
			pH 6.7		
AM0	6	17.9	3.3	21.2	1.010
AM+	100	12.9	2.4	15.2	0.770
mean	53	15.4	2.9	18.2	0.890
			F-test		
AM	**	NS	NS	NS	NS
pH	NS	*	*	*	NS
($LSD_{0.05}$)		(3.9)	(0.8)	(4.5)	
AMxpH	NS	NS	NS	NS	NS

AM = arbuscular mycorrhizal fungi, pH = soil pH, * = significant at $P < 0.05$, NS = non-significant, the numbers in parenthesis are least significant difference at $p<0.05$, # = data was arsine transformed before analyzed.

Table 4.8 Effect of AMF and soil acidity shoot P and N concentration, total P and N content of cowpea applied with N fertilizer or inoculated with rhizobium

	Nodule/pot	Shoot P concentration (%)	Total P content (mg/pot)	Shoot N concentration (%)	Total N content (mg/pot)
			pH 5		
AM0	552	0.45	55.3	3.77	520.3
AM+	404	0.49	57.9	3.72	440.7
Mean	487	0.47	56.6	3.75	480.5
			pH 6.7		
AM0	652	0.39	82.0	3.55	711.0
AM+	466	0.51	77.9	3.99	567.1
mean	559	0.45	79.9	3.77	639.1
			F-test		
AM	**	NS	NS	NS	NS
($LSD_{0.05}$)	(117)				
pH	NS	NS	**	NS	*
($LSD_{0.05}$)			(14.3)		(146.9)
AMxpH	NS	NS	NS	NS	NS

AM = arbuscular mycorrhizal fungi, pH = soil pH, * = significant at $P < 0.05$, NS = non-significant, the numbers in parenthesis are least significant difference at $P < 0.05$

4.3.3 Comparing effect of soil acidity on cowpea growth varied with soil P level

Root colonization was not found in this experiment

Cowpea growth

Phosphorus application increased shoot dry weight in both soil pH levels. But the response to P was less in acid soil. In another way, to get same shoot dry weight cowpea in acid soil needed more P application than in non-acid soil. From the data in Table 4.10, cowpeas in non-acid soil had 13.6 g/pot shoot dry weight when they were applied with 33 kg P/ha but in acid soil they need 45 kg P/ha to get the same shoot weight. Soil acidity depressed shoot dry weight in every P level but the effect of soil acidity was distinct in range between P16 to P45. And the biggest impact of soil acidity was found at P33 (Table 4.10).

Same as shoot weight P application increased root dry weight in both soil pH levels. The response to P was less in acid soil. Soil acidity depressed root dry weight only when P level was lower than 82 kg P/ha. At P82 root dry weight was not affected by soil acidity. The impact of soil acidity was found in P level ranging from 9 – 45 kg P/ha and the effect was biggest at P33 (Table 4.10).

Same as shoot and root weight P application increased total dry weight in acid and non-acid soil. The response to P was less in acid soil. Soil acidity depressed total dry weight in every P level but the effect was distinct between P16 to P45 and it was biggest at P33 (Table 4.9).

Nodulation

There was no interaction between P level and soil pH on nodule number. Soil acidity had no effect on nodule number. Nodule number only depended on P level. Applying P increased nodule number in both soil pH levels (Table 4.10). But for nodule dry weight the interaction between P and soil pH was found. Phosphorus application increased nodule dry weight in both soil pH. But the response to P was less in acid soil especially in low P level. For example, increasing P level from P9 to P16 increased nodule dry weight 136% in non-acid soil but in acid soil there was on response. To increase nodule dry weight in acid soil cowpea needed at least 33 kg P/ha (P33) to increased nodule dry weight for 260% (compare with P9) while in non-acid nodule weight in P33 was higher than P9 for 368%. The effect of soil acidity on nodule dry weight depended on P level. At lowest P level (P9), soil acidity had no effect on nodule dry weight. Soil acidity depressed nodule dry weight in P16 or above. The biggest impact of soil acidity on nodule dry weight was found in P33 (Table 4.10).

Nutrient status in plant

Applying P increased shoot P concentration in both soil pH. Soil acidity depressed shoot P concentration in every P level (Table 4.11). The positive correlation between shoot P concentration and shoot dry weight was found in both soil pH. When shoot P concentration was high, Plant had higher shoot growth (Figure 4.1). Applying P increased total P content in both soil pH but the response was less in acid soil. Increasing P application rate from P9 to P33 did not increase P content in acid soil. To increase P content in acid soil at less P45 application rate was needed. In non-

acid soil total P content was continually increased when P level increased from P9 to P82. Soil acidity depressed P content in all P levels except at P9 (lowest P level). The biggest impact of soil acidity on P content was found in P33 (Table 4.11).

Opposite with shoot P, shoot N concentration was depressed by P application in both soil pH levels. Soil acidity had no effect on it (Table 4.11). The negative correlation between shoot N concentration and shoot dry weight was found. Plant that had higher shoot growth had less N accumulation in shoot (Figure 2). Phosphorus application increase total N content in both soil pH but the response was less in acid soil. Soil acid depressed N content in all P levels except in P9 and the biggest effect of soil acidity was found in P33 (Table 4.11).

Table 4.9 Soil P concentration (mg P/kg) in varying P application rate of acid (pH 5) and non-acid soil (pH6.7)

Soil pH	P application (kg P/ha)				
	9	16	33	45	82
pH5	4.9 ± 0.5	7.0 ± 0.2	10.8 ± 0.1	16.2 ± 0.2	29.8 ± 0.4
pH6.7	5.8 ± 0.1	7.7 ± 0.4	11.7 ± 0.1	16.5 ± 0.8	30.9 ± 0.3

The numbers following the symbol ± are standard error

Table 4.10 Effect of P application treatment on biomass yield and nodulation of cowpea in acid (pH 5) and nonacid (pH6.7) soil

P treatment	Shoot dry weight (g/pot)	Root dry weight (g/pot)	total dry weight (g/pot)	nodule dry weight (mg/pot)	Nodule number per pot
			pH5		
P9	2.6 g	1.1 e	3.7 g	34 g	128
P16	3.8 fg	1.7 de	5.4 fg	71 fg	157
P33	5.8 f	2.2 d	7.8 f	123 ef	196
P45	14.2 d	4.0 b	18.2 d	381 d	314
P82	25.6 b	6.1 a	31.8 b	836 b	558
			pH6.7		
P9	4.8 fg	2.1 d	6.9 f	73 fg	134
P16	8.3 e	3.2 c	11.5 e	173 e	254
P33	13.6 d	4.2 b	17.8 d	342 d	288
P45	20.1 c	5.7 a	25.8 c	510 c	378
P82	29.5 a	5.8 a	35.3 a	994 a	515
			F-test		
P	**	**	**	**	*
($LSD_{0.05}$)	(1.6)	(0.6)	(2.0)	(57)	(80)
pH	**	**	**	**	NS
($LSD_{0.05}$)	(1.0)	(0.4)	(1.3)	(36)	
pHxP	**	**	*	*	NS
($LSD_{0.05}$)	(2.3)	(0.8)	(2.9)	(81)	

Means in the same column followed by different letter are significant different at $P < 0.05$, P = phosphorus level, pH=soil pH, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, NS = non-significant, the numbers in parenthesis are Least Significant Difference at $P < 0.05$ ($LSD_{0.05}$)

Table 4.11 Effect of P application treatment on shoot P and N concentration, total N and P content and P uptake efficiency of cowpea in acid (pH 5) and nonacid (pH6.7) soil

P treatment	Shoot P concentration (%)	Total P content (mg/pot)	P efficiency (mg/g)	Shoot N concentration (%)	Total N content (mg/pot)
			pH 5		
P9	0.071	3.4 f	2.4 g	2.72	99 f
P16	0.080	4.7 f	2.8 ef	2.62	126 f
P33	0.084	7.1 ef	3.2 e	2.34	164 f
P45	0.086	16.8 d	4.2 cd	2.16	367 d
P82	0.091	30.4 b	5.0 b	2.10	630 b
			pH 6.7		
P9	0.075	5.7 f	2.7 fg	2.41	148 f
P16	0.083	10.3 e	3.2 e	2.32	242 e
P33	0.090	17.1 d	4.1 d	2.34	383 d
P45	0.093	25.6 c	4.5 c	2.22	539 c
P82	0.105	38.4 a	6.6 a	2.09	697 a
			F-test		
P	**	**	**	*	**
($LSD_{0.05}$)	(0.005)	(2.4)	(0.3)	(0.18)	(41)
pH	**	**	**	NS	**
($LSD_{0.05}$)	(0.003)	(1.5)	(0.2)		(26)
PxpH	NS	*	**	NS	**
($LSD_{0.05}$)		(3.4)	(0.4)		(58)

Means in the same column followed by different letter are significant different at $P < 0.05$, P = phosphorus level, pH = soil pH, * = significant at $P < 0.05$, ** = significant at $P < 0.01$, NS = non-significant, the numbers in parenthesis are Least Significant Difference at $P < 0.05$ ($LSD_{0.05}$)

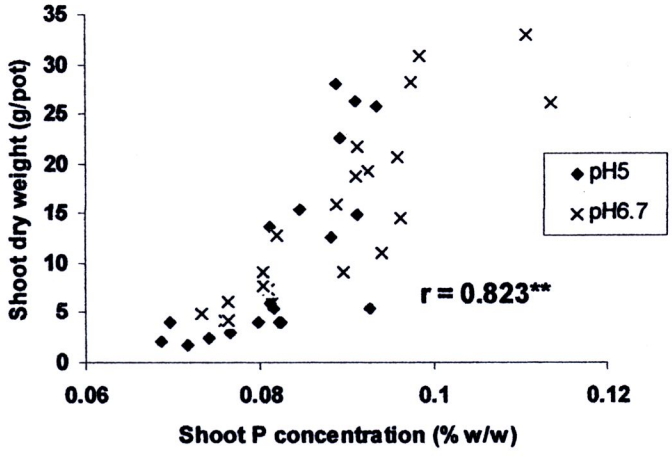

Figure 4.1 Correlation between shoot P concentration and shoot dry weight in acid and non-acid soil. r = correlation coefficient calculated from both soil pH levels, ** = significant at $P < 0.01$

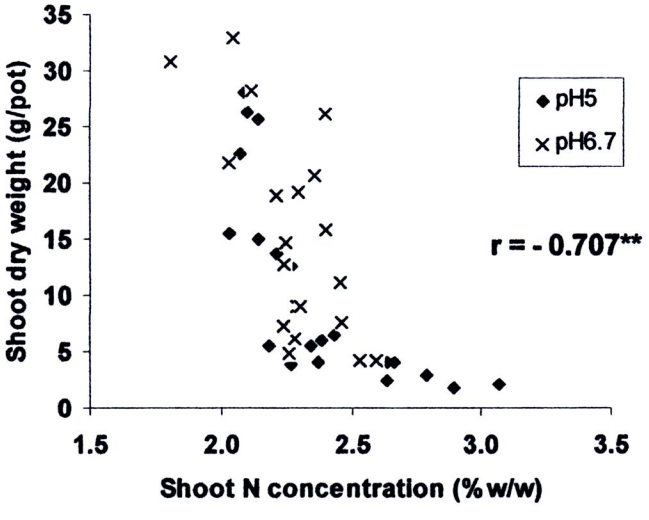

Figure 4.2 Correlation between shoot N concentration and shoot dry weight in acid and non-acid soil. r = correlation coefficient calculated from both soil pH levels, ** = significant at $P < 0.01$

4.4 Discussion

In the experiment 4.2.1, soil acidity depressed shoot growth and nodule formation (Table 4.2 to 4.6). The AMF could not alleviate acid soil stress. Because of very low % root colonization, AMF not only had no benefit on cowpea growth and nodulation but also depressed shoot growth and nodule biomass (Table 4.2and 4.5). Cowpeas in all treatments exhibited P deficiency symptom such as chlorosis and brown spot at 20 days after sowing. Phosphorus deficiency should be the limiting factoring in this experiment. Benefit of AMF on host plant highly depended on compatibility between fungi and host plant (Skipper and Smith, 1979: Boddington and Dodd, 1998) and the environment factors especially soil P (Bethlenfalvay, 1992).

In the experiment 4.2.2 the commercial inoculum was used. In the early stage (20 days after sowing) cowpea expressed P deficiency symptom same as in experiment 4.2.1. This result suggested that the problem might be too low soil P not the compatibility between AMF and host plant. Bethlenfalvay, (1992) suggested that in soil with extremely low P, competition for P between host plant and AMF can cause growth depression of host same as the experiment 4.2.1. Therefore at 23 days, 228 mg P (inform of KH_2PO_4) was added to every pot. In several days after application, the P deficiency symptom disappeared. When plants were harvested at 45 days (pod filling stage) AMF still had no benefit on host same as previous experiment. But in this experiment no P deficiency symptom was found at harvest. The shoot P in Table 4.8 show P status in cowpea was above the critical level (0.149%) reported by Wan Othman *et al* (1991). Applying P at 23 days caused sufficient P level in cowpea. When plants are supplied with enough P, AMF colonization normally has no benefit on legume host and it might limit growth and

nodulation because of carbon source competition (Bethlenfalvay,1992). This is the reason why AMF had no benefit on cowpea in this experiment. The too low soil P in experiment 4.2.1 and to high soil P in experiment 4.2.2 indicted that P application rate is very important of testing effectiveness of AMF. The suitable P levels were find in experiment 4.2.3.

In experiment 4.2.3 shoot P concentration in every treatment was lower than 0.149% (Table 4.11) which is the critical level reported by Wan Othman *et al* (1991). And biomass yield increased proportionally with shoot P concentration (Figure 4.1). These evidences indicated that P deficiency was the limiting factor for cowpea growth in this experiment. Therefore applying P fertilizer enhanced cowpea growth in both soil pH. But P deficiency in acid soil was more serious than in no-acid soil. Cowpea in acid soil needed more P than in non-acid soil to get the same growth (Table 4.10). Soil acidity depressed cowpea growth by accentuating P deficiency. The evidence was shown by P status in cowpea that soil acidification depressed P status (shoot P concentration Table 4.11) and to total P uptake (total P content Table 4.11). In acid soil P become less available for plant because phosphate ions in soil solution were more absorbed by soil colloid or boned with aluminum ion in acid soil and become less available for plant (Haynes, 1982). It was supported by the result that available P in acid was less than in non-acid soil (Table 4.9). There is a doubt that which legume and its N_2 fixation system are more sensitive to soil acidity. If the N_2 fixing system is more sensitive N deficiency must be the limiting factor when legumes are grown in acid soil. But in this experiment soil acidity had no effect on N status in cowpea (Table 4.11). And the dilution effect on N was found. When growth was enhanced by applying P plant had lees N accumulation (Table 4.10 and 4.11). Plant had lees N

concentration when plant had higher growth (Figure 4.2). These indicated that N deficiency was not the limiting factor in this experiment. But this is not the general conclusion for all legumes in acid soil. Cline *et al.* (1991) reported that N fixation is more sensitive to soil acidity than soybean and N deficiency is the limiting factor of soybean when grown in acid soil. Glenn and Dilworth (1991) suggested that it difficult to make a general conclusion because there is the variation in both rhizobium and legumes in their tolerance to complicate adverse effects in acid soil. In this experiment, the effect of acid soil highly depended on P level. Testing the effectiveness of AMF to alleviate acid soil stress in next experiment should be conducted in P levels that impact of soil acidity on cowpea growth is high. The impact of soil acidity on cowpea growth and nodulation was high in range from P16 to P45 and the biggest impact was found in P33 (Table 4.10). At lowest (P9) or highest (P82) P level the adverse effect of soil acidity was less. Another concern is the suitable P level for mycorrhiza because the symbiosis is highly depends on soil P level (Marschner, 1995). Brundrett *et al.* (1996) suggested that P level that provides 60% of maximum plant growth is suitable for best response to AMF. The biomass yield in P16, P33 and P45 were 25, 38 and 66% of biomass yield in P82 (highest P level). Therefore testing the effectiveness of AMF to alleviate acid soil stress should be conducted in P16, P33 and P45.

At too low and too high soil P in experiment 4.2.1 and 4.2.2 respectively there was no benefit of AMF on cowpea growth. The adverse effect of soil acidity was biggest when P application rate was in range 16, 33 and 45 kg P/ha. These 3 levels of P application were chosen to test the effectiveness of AMF to alleviate acid soil stress in cowpea in chapter 5.