African Journal of Biotechnology Vol. 10(65), pp. 14410-14418, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1300
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Alleviating acid soil stress in cowpea with a local population of arbuscular mycorrhizal fungi

Ayut Kongpun[1]*, Bernard Dell[2] and Benjavan Rerkasem[1]

[1]Division of Agronomy, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand.
[2]Sustainable Ecosystems Research Institute, Murdoch University, Perth, Western Australia, Australia.

Accepted 13 July, 2011

In Huai Teecha village in Northern Thailand, local cowpeas were grown on acidic low phosphorus soil without stress symptoms. Arbuscular mycorrhizal fungi from this system have been found to promote growth of many crops but there is no information about their benefit in cowpea. In a field experiment, three improved cowpea lines (ITD - 1131, Ubon Ratchathani and IT90K – 227 - 2) and a local line (Teecha 1) were grown in 3 farmer's fields on acid low P soils. Roots of the cowpea lines were all heavily colonized by the fungi and their leaf P was within the sufficient range. In a pot experiment, the cowpea line Ubon Ratchathani was grown in acidic and non acidic (pH 5 and 6.7, respectively) soil with three rates of phosphorus (50, 104 and 141 mg phosphorus pot^{-1}) with and without arbuscular mycorrhizal fungi inoculation. Total dry weight of inoculated cowpea was not affected by soil acidity while it was depressed in un-inoculated plants. The fungi increased total dry weight at 50 and 104 mg phosphorus ha^{-1} but had no effect at 141 mg phosphorus pot^{-1}. Therefore, the fungi had been shown to enhance P uptake by cowpea roots, which resulted in direct benefit to cowpea growth in acidic low P soil.

Key words: Mycorrhiza, cowpea, acid soil.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is a multi-purpose legume crop that is widely grown throughout the world's tropical and sub-tropical regions, where it is grown by subsistence farmers to provide vegetables, from its leaves and fresh fruit, fodder and dry seed (Steele et al., 1985). In Africa, cowpea is valued by farmers because of its relatively short duration (Adjei-Nsiah et al., 2007).

In association with nitrogen (N) fixing nodule bacteria, a cowpea crop may also provide an input of 50 to 80 kg N ha^{-1} into the soil on average (Peoples et al., 2009), and up to 60 kg N ha^{-1} by one estimate in Ghana (Dakora et al., 1987). However, soil acidity is a major limiting factor for nitrogen fixation by legumes as well as for their production (Craswell and Pushparajah, 1989; Edwards et al., 1981; Zahran, 1999). Acidic soils occupy around 40% of the world's potentially arable lands (Von Uexküll and Mutert, 1995).

In Southeast Asia, acid upland soils occupy 64% of the total land (International Plant Nutrition Institute, 2009). In acidic soils, legume growth may be inhibited by a combination of factors, including toxicity of cations such as aluminium (Al), manganese (Mn) and hydrogen ion (H^+) and deficiency of essential elements such as calcium (Ca), magnesium (Mg), phosphorus (P) and molybdenum (Mo) (Marschner, 1995), with P being the most common (Maddox and Soileau, 1991).

The common solutions for acidic soil problem in legumes are liming (Piedra and Munns, 1990; Arshad and Gill, 1996; Edwards et al., 1981) or P fertilizer application (Haynes and Ludecke, 1981; Hafner et al., 1992).

However, sometimes the cost or logistics of applying these chemicals may be prohibitive or surface application

*Corresponding author. E-mail: ayutkongpun@yahoo.com. Tel: 66897579334. Fax: 66 53 210000.

Abbreviations: P, Phosphorus; **AMF,** arbuscular mycorrhizal fungi; **YFEL,** youngest full expanded leaves; **LSD,** least significant difference; **P1**, **P2 and P3**, applying phosphorus 50, 104 and 141 mg P pot^{-1}, respectively; **AM+,** inoculating with arbuscular mycorrhizal fungi; **AM0,** un-inoculated treatment; **DW,** dry weight; **N,** nitrogen.

can not solve subsoil acidity (Parkpian et al., 1991). On acidic low P upland soil in northern Thailand, farmers grow many legumes including cowpea without stress symptoms being expressed. In this system of shifting cultivation, upland rice is grown together with 30 species of crops including several legumes in rotation with 6 years of fallow, with very little external inputs, and soil fertility is maintained by a fallow enriching tree, *Macaranga denticulata* (Yimyam et al., 2003).

Nutrient accumulation and recycling by *M. denticulara*, on the other hand, is highly dependent on its symbiotic association with naturally occurring arbuscular mycorrhizal fungi (AMF) (Youpensuk et al., 2005b). This diverse and abundant native population of arbuscular mycorrhizal fungi; AMF has been shown to directly improve growth of upland rice and other crops in the system, including job's tears and sorghum (Wongmo 2008), as well as tree crops such as rubber, coffee and citrus (Yimyam et al., 2008). It is possible that cowpea growing on acidic low P soil may also directly benefit from association with AMF, which are well known for their role in improving P nutrition of the host plant (Marschner, 1995).

The objective of the field study was to assess the P status and mycorrhizal root colonization in cowpea growing on acidic, low P soil in farmers' fields known for their naturally abundant and diverse native population of AMF. This was followed by a pot experiment that measured the effectiveness of AMF from the field on nutrient uptake and growth of cowpea.

MATERIALS AND METHODS

This study consisted of a field study and a pot experiment in using an autoclaved soil.

Field experiment

The field study was conducted in wet season (May to July 2008) in fields belonging to 3 farmers at Haui Teecha village, northern Thailand (19° 78' N, 93° 84' E, altitude 800 m). Available soil P (Bray II), soil pH and AMF spore density in the soil of each farmer's field are shown in Table 1. Three improved cowpea lines (provided by Ubon Ratchathani Field Crop Research Centre) including ITD-1131 (sensitive to acid soil), cv. Ubon Ratchathani (moderately sensitive), IT90K-227-2 (tolerant) and a local variety from within the village (Teecha 1) were grown with 3 replications in each field, where they were arranged in a randomized complete block design. A 50 × 50 cm plot was an experimental unit and 8 plants were sown in each plot. The pH tolerant line was classified by yield depression when soil pH decreased from 6.7 to 5. Yield of the tolerant line was not affected by the soil acidification while the yield of moderately sensitive and sensitive lines was depressed 22% and 26%, respectively.

At 50 days after sowing roots of 2 plants in each plot were excavated to measure AMF root colonization, two soil cores (4.5 cm diameter × 15 cm depth) were taken from the base of 2 plants in each plot and combined. These samples were used for soil analysis and spore extraction. Youngest full expanded leaves (YFEL) of 4 plants in each plot were collected for the determination of P concentration. The leaves were oven dry for 72 h before grinded. Zero point two gram of grinded sample was dried ash at 550°C for 8 h. Phosphorus was extracted by 2 ml of 1:1 H_2O : concentrated HCl before P concentration was measured by the molybdovanadate-phosphoric acid method (Murphy and Riley, 1962).

The root samples were cut into 1 to 2 cm pieces, cleared in 10% KOH for several days before staining with 0.05% trypan blue in lactoglycerol. 50-30 pieces of root fragment (to reach 30 cm of total root rang) were randomly taken from each sample and placed on a microscopy slide. Root colonization percentage was assessed using the intercept method (Brundrett et al., 1996) under a compound microscope. Moisture content of each soil sample was measured. Thirty gram from each soil sample was used for spore extraction by wet sieving and sucrose centrifugation (Brundrett et al., 1996). The remainder was air dried and soil pH (1:1, H_2O) and Bray II P was determined.

Data were analyzed using analysis of variance and different amount of treatments were compared using least significant different (LSD) value ($p \leq 0.05$). The data in percentage were transformed by arcsine before analysis.

Pot experiment

Inoculum production

Soil (pH 4.9, 3.8 mg P kg^{-1}) from the root zone of the fallow enriching tree of *M. denticulata* was collected from Haui Teecha in the wet season, and contained 4 AMF spores g^{-1}. Spineless mimosa *(Mimosa invisa)* was grown in this soil as a host plant to multiply the spores in pots for 8 months. This mimosa root zone soil, in which the number of spores was increased to 25 spores g^{-1} was used as soil inoculum in the pot experiment.

Growth medium preparation

Plant growth medium was prepared from a mixture of sand and soil. Sansai soil (0 to 30 cm depth), which contained 3.8 mg P kg^{-1} (Bray II) and pH 5.9 (1:1 H_2O), was collected from Mae Hia Agricultural Research Station and Training Center, Chiang Mai University. 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 samples were placed in plastic bags. The mixture was adjusted to pH 5 (acid soil) with $Al_2(SO_4)_3$ $18H_2O$ or pH 6.7 (non acid soil) with $CaCO_3$. Basal nutrients were applied (in mg kg^{-1}) as follows: 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 medium was autoclaved at 121°C for 1 h.

Experimental arrangement

The pot experiment was conducted in a glasshouse at Chiang Mai University in the dry season (26 January to 12 May). The experiment was designed as a factorial of 3 factors, arranged in a randomized complete block with 4 replications. A 5 L plastic pot (20 cm diameter) containing 3.6 kg growth medium was one experimental unit. The factors were soil pH (acidic soil pH 5 and non-acidic soil pH 6.7), P (50, 104 and 141 mg P pot^{-1}, designated P1, P2 and P3, respectively) and AMF inoculation (inoculated, AM+; and uninoculated, AM0). The P treatments were applied in form of KH_2PO_4. Seeds of cowpea (cv. Ubon Ratchathani) were surface sterilized with 70% ethanol for 5 min then washed three times with sterilized water before sowing five seeds per pot. Each

Table 1. Soil pH, soil P concentration and spore density in topsoil (0 to 10 cm) of 3 farmer's fields.

Farmer	Soil pH (1 : 1 H_2O)	Bray II soil P concentration (mg P kg^{-1})	AMF spore density (spores g^{-1} soil)
Tongdee	5.65 ± 0.06	2.20 ± 1.03	6.94 ± 1.79
Kayo	5.13 ± 0.08	0.74 ± 0.06	3.01 ± 1.25
Luyo	5.08 ± 0.05	2.79 ± 0.42	2.83 ± 0.18

The values are mean of 3 replications ± standard error.

Table 2. Root colonization, spore density in root zone soil and P concentration in YFEL of 4 cowpea cultivars grown in Teecha at 50 days after sowing.

Cowpea cultivar	Farmer's field		
	Tongdee	Kayo	Luyo
Root colonization (%)			
ITD-1131	85.5	85.3	82.1
Ubon Ratchathani	90.0	90.5	78.9
IT90K-227-2	73.2	85.0	89.1
Teecha1	81.7	87.0	85.5
F-test	NS	NS	NS
Spore density in root zone (spore g^{-1})			
ITD-1131	8.43	9.65	7.51
Ubon Ratchathani	20.69	10.66	3.90
IT90K-227-2	21.02	12.22	4.35
Teecha1	11.35	11.39	9.33
F-test	NS	NS	NS
P concentration in YFEL (% w/w)			
ITD-1131	0.27	0.32	0.32
Ubon Ratchathani	0.30	0.29	0.32
IT90K-227-2	0.26	0.31	0.38
Teecha1	0.23	0.29	0.31
F-test	NS	NS	NS

NS = Not significant Values are means of 3 replications.

seed was inoculated with 1 ml of appropriate rhizobium suspension at 10^9 cells ml^{-1} (the suspension was provided by the Department of Soil Science, Faculty of Agriculture, Chiang Mai University).

The AM+ treatment consisted of 50 g of soil inoculum pot^{-1} and AM0 treatment was the same weight of AM+ autoclaved at 120°C for 1 h. Pots were watered every day. At 10 days after sowing, plants were thinned to 3 plants per pot. Plants were harvested at 50 days after sowing (at pod filling stage). Shoots were cut at ground level then oven dried at 75°C for 48 h before weighing.

Roots were washed free of soil. Root nodules were collected and oven dried before weighing. Fresh roots were weighed before cutting into approximately 1 cm pieces. A root sub-sample (10% of total root fresh weight) was randomly taken from every pot for AMF measurement. The remaining roots were oven dried before weighing. Assessment of root colonization was carried out in the same way as samples from the field study. Phosphorus concentration in plant tissue (shoot and root) was measured by the Molybdovanadate-Phosphoric Acid method (Murphy and Riley, 1962) as described before.

Phosphorus uptake per unit root weight (total plant P/root dry weight) was used as an indicator for P uptake efficiency. Data were analyzed using analysis of variance and differences among treatments were compared using least significant difference (LSD) value ($p \leq 0.05$). The data in percentage were transformed by arcsine before analysis.

RESULTS AND DISCUSSION

Field experiment

The field study did not find any difference among the 4 cowpea lines in their association with the native AMF arbuscular mycorrhizal fungi; population, as indicated by root colonization, which ranged from 73 to 90% and rhizosphere spore density of 9.7 to 12.2 spores g^{-1} soil (Table 2). The cowpea genotypes were thus equally able to be infected and propagate with the local AMF

Table 3. Root colonization in AM+ cowpea (cv. Ubon Ratchathani) grown in acid and non-acid soil applied with 3 levels of P fertilizer in pot culture experiment at pod filling stage (50 days old).

Soil pH (1 : 1 H_2O)	Root colonization (%)		
	P1	P2	P3
pH 5 (acid soil)	45.7	40.4	44.4
pH 6.7 (non-acid soil)	56.8	53.0	46.6
F-test	pH^{NS}	P^{NS}	$PxpH^{NS}$

NS = Not significant values are means of 4 replications, pH = soil pH, P = phosphorus level.

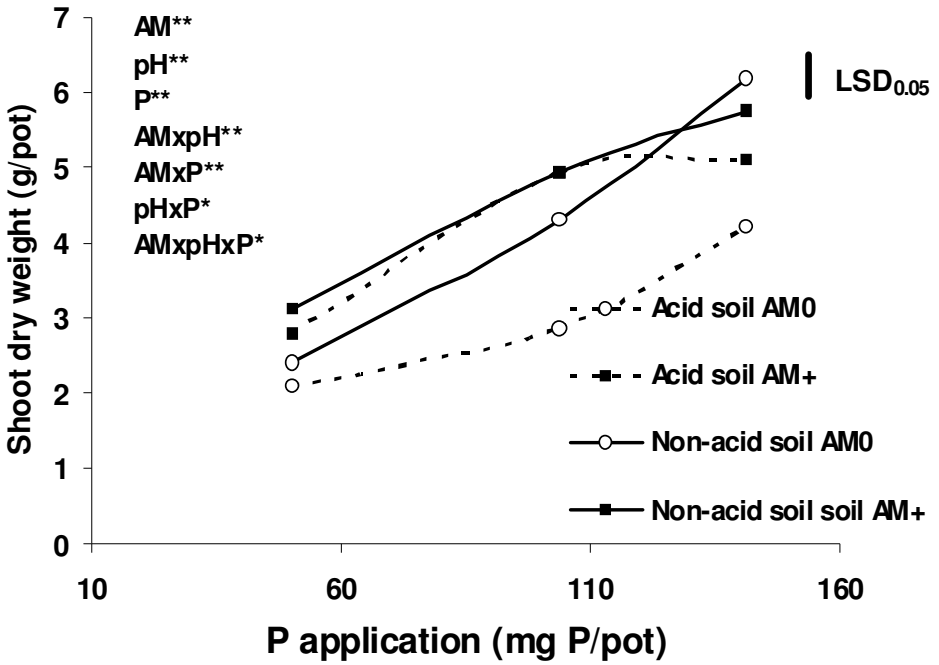

Figure 1. Effect of AMF inoculation on shoot dry weight in acid and non- acid soil as a function of P fertilizer. AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, * = F-test significant at $p < 0.05$, ** = F-test significant at $p < 0.01$, the LSD bar is for the 3 way interaction.

population in Haui Teecha. This is in accordance to a previous study (Rajapakse et al., 1989) reporting a high degree of specificity on cowpea genotype in root colonization by a pure culture of *Glomus fasciculatum*. The AMF population associated with the shifting cultivation system at Haui Teecha is highly diverse. Classified by their spore type, 29 species in 6 genera were found in the rhizosphere of *M. denticulata* (Youpensuk et al., 2004) and 17 species in 5 genera in the rhizosphere of upland rice (Youpensuk et al., 2005a) in farmer's fields of this village.

The high diversity of AMF in this system made each cowpeas having chance to associate with the AMF that they prefer. Therefore root colonization was not depended on cowpea genotypes as previous experiment that used pure culture inoculum. In spite of the very low available soil P in the field (0.7 to 2.8 mg P kg^{-1}), the youngest full expanded leaves P (YFEL P) in the cowpea ranged from 0.23 to 0.34% (Table 2), within the critical level of 0.23% for 90% maximum biomass yield (Ikombo, 1991). It seems that cowpea growing in low P acidic soil may have directly benefited from the local AMF, which was confirmed in the pot experiment.

Pot experiment

In the pot experiment, roots of un-inoculated cowpea (AM0) were not infected by AMF, confirming that there was no contamination in the un-inoculated control. In AM+ plants, soil pH had no effect on root colonization, which ranged from 40 to 68% (Table 3). The effect of AMF on shoot dry weight varied with both soil acidity and P level (Figure 1).

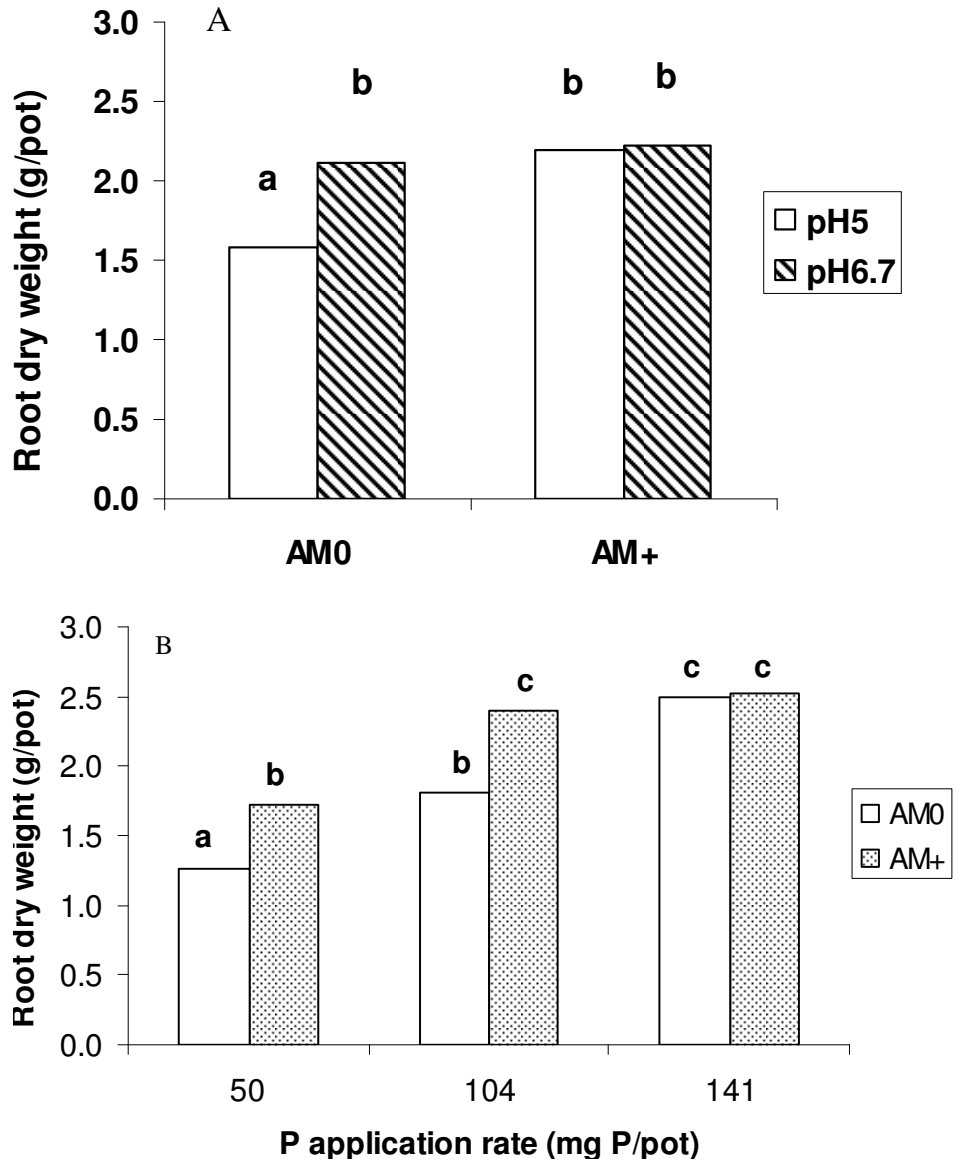

Figure 2. Interaction between AMF inoculation and soil pH (A) and between AMF inoculation and soil P level (B) on root dry of cowpea (cv. Ubon Ratchathani) at pod filling stage. Different lower cases indicate significant difference at $p < 0.05$. AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, * = F-test significant at $p < 0.05$, ** = F-test significant at $p < 0.01$.

In the acidic soil, AMF enhanced shoot growth at all 3 P levels, with the biggest effect at P2. In non acidic soil, the response to AMF was less and detectable only at P1. In acidic soil, cowpea should get more benefit from AMF which is well known for improving P acquisition of host plant (Marschner, 1995) because soil acidity accentuates P deficiency (Harter, 2002). There was no 3-way interaction on root dry weight but there were 2-factor interaction of all combinations. For the interaction between soil pH and AMF, soil acidity depressed root dry weight of uninoculated plants (AM0) while root weight of inoculated plants (AM+) was not affected (Figure 2A) indicating the positive effect of mycorrhiza on alleviating acid soil stress. For the interaction between AMF and soil P level, AMF increased root weight in low (P1) and medium (P2) P levels but not at the high P level (P3) (Figure 2B).

In the presence of high P level, the effectiveness of mycorrhiza is therefore, lowered. The response to AMF in total dry weight was similar to that of root dry weight (Figure 3). These results indicate that shoot growth was more sensitive to AMF than root growth. Therefore, shoot dry weight should be a better indicator for AMF response than root or total dry weight. This phenomenon is

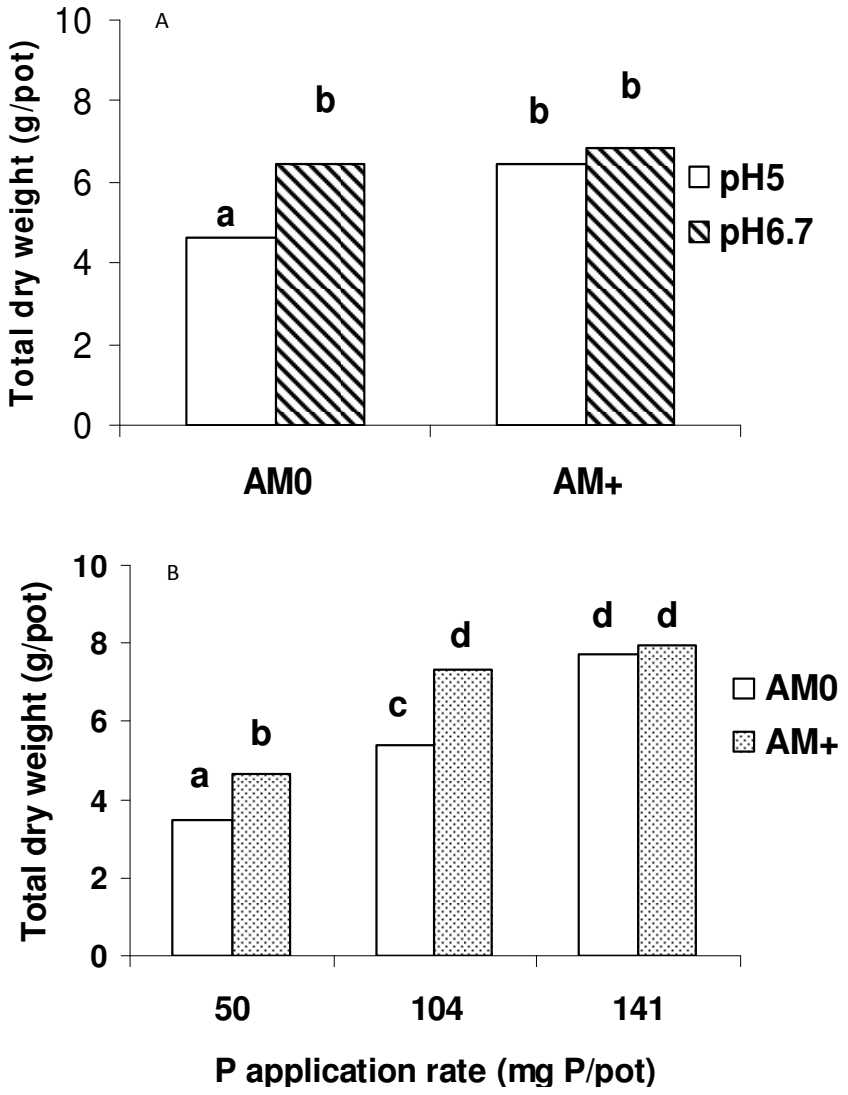

Figure 3. Interaction between AMF inoculation and soil pH (A) and between AMF inoculation and soil P level (B) on total dry of cowpea (cv. Ubon Ratchathani) at pod filling stage. Different lower cases indicate significant difference at $p < 0.05$. AM = arbuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, * = F-test significant at $p < 0.05$, ** = F-test significant at $p < 0.01$.

common in plants associated with AMF, leading Marschner (1995) to suggest that competition for photosynthates between the root and fungi could cause root growth to have less response to AMF than shoot growth.

When shoot dry weight was used as a growth indicator and shoot P concentration was used as a measure of the P status of the plant, a strong positive correlation ($r = 0.706$, $P < 0.01$) between growth and P status was found (Figure 4). Moreover, the shoot P concentration in all treatments was lower than 0.149% (Table 4), which is the adequate level for cowpea (Othman et al., 1991). These results confirm that P was the limiting factor for cowpea growth in this experiment. Shoot P concentration was increased significantly by liming, P application or inoculation with AMF (Table 4). Liming to raise soil pH is a way to improve P availability in acidic soil.

In acidic soil, Al^{3+} is released from unreactive $Al(OH)_3$ into the soil solution (Harter, 2002), and can bind with phosphate ions to make P unavailable to plants. When soil pH is lifted, Al^{3+} is immobilized and P availability is improved (Bolt, 1979). With linear increase in shoot P concentration with increasing rate of P application ($r = 0.999$, $P < 0.01$), AMF inoculation had about the same effect as application of 41 mg P kg^{-1} soil. The effect of AMF in enhancing P uptake of cowpea was also evident in P uptake; efficiency measured as total plant P content per unit root dry weight (Table 5). On the average, the

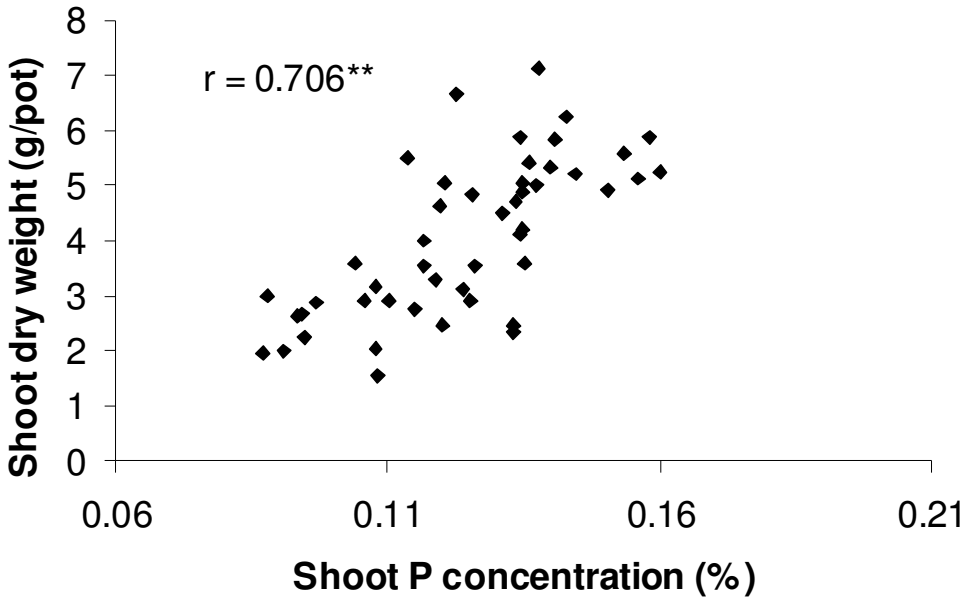

Figure 4. Relationship between shoot P concentration and shoot dry weight in pot experiment. r = correlation coefficient, ** = significant at p < 0.01.

Table 4. Effect of AMF and P application on shoot P concentration (% w/w) of cowpea (cv. Ubon Ratchathani) at pod filling stage (50 days old) in acid and non-acidic soil.

Variable	Acidic soil				Non-acidic soil		
	AM0			AM+	AM0		AM+
P1	0.097			0.121	0.094		0.125
P2	0.108			0.129	0.121		0.141
P3	0.124			0.141	0.139		0.147
F-test	AM**	pH**	P**	AMxpH^{NS}	AMxP^{NS}	pHxP^{NS}	AMxpHxP^{NS}
$LSD_{0.05}$	0.006	0.006	0.007	-	-	-	-

AMF= Abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at p < 0.01, Values are means of 4 replications.

Table 5. Effect of AMF and P application on P uptake per unit root dry weight (mg P g^{-1} root DW) of cowpea (cv. Ubon Ratchathani) at pod filling stage (50 days old) in acid and non-acidic soil.

Variable	Acidic soil				Non-acidic soil		
	AM0			AM+	AM0		AM+
P1	2.76			3.58	2.77		4.31
P2	3.28			4.25	3.63		5.20
P3	3.80			4.97	4.11		5.21
F-test	AM**	pH**	P**	AMxpH^{NS}	AMxP^{NS}	pHxP^{NS}	AMxpHxP^{NS}
$LSD_{0.05}$	0.24	0.24	0.29	-	-	-	-

AMF = Abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at p < 0.01, Values are mean of 4 replications.

roots of AM+ cowpea took up 4.59 mg P g^{-1} root DW compared with 3.39 mg P g^{-1} root DW in AM0. Inoculating with AMF increased nodule dry weight in both soil pH but the effect was bigger in acid soil (Table 6).

In AM0, applying P depressed shoot N concentration but in AM+, shoot N was stable along P levels (Table 7). The shoot N concentration was diminished by higher shoot growth when plants were applied with P (Figure 1;

Table 6. Effect of AMF and P application on cowpea nodule dry weight (mg pot^{-1}) (cv. Ubon Ratchathani) at pod filling stage (50 days old) in acidic and non-acidic soil.

Applied P	Acid soil		Non-acid soil		P Mean
	AM0	AM+	AM0	AM+	
P1	10	96	23	102	58 c
P2	74	238	132	244	172 b
P3	184	297	257	264	250 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	- -	-

AMF = abuscular mycorrhizal fungi, pH = soil pH, P = phosphorus level, NS = non-significant, ** = significant at p < 0.01, * = significant at p, 0.01, Values are mean of 4 replications, 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.

Table 7. Effect of AMF and P application on shoot N concentration (% w/w) of cowpea (cv. Ubon Ratchathani) at pod filling stage (50 days old) in acidic and non-acidic soil.

Variable	Acid soil			Non-acid soil		Average	
	AM0		AM+	AM0	AM+	AM0	AM+
P1	3.25		2.44	3.15	2.57	3.20 a	2.51 bc
P2	2.57		2.60	2.31	2.67	2.44 bc	2.64 bc
P3	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	-	-

AMF = 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.

Table 7). This result indicates 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.

Benefits from AMF in the promotion of nutrient uptake and plant growth is also realized by inoculating with AMF spores extracted from the rhizosphere of the host plant (Youpensuk et al., 2006; Kittiworawat et al., 2010). However, the use of soil from the root zone of the host plant as an AMF inoculum has a clear practical advantage in the ease of preparation. Indeed, soil from the root zone of the fallow enriching tree *M. denticulata* has been effectively used as AMF inoculum on seedlings of rubber (Kanyasone, 2009) and coffee (Yimyam, 2006) as well as in field crops (Wongmo, 2008). Just as we have shown with cowpea, the soil inoculum collected from the root zone of *M. denticulate*, that had been multiplied in the rhizosphere of *Mimosa invisa*, was also effective in promoting growth and nutrient uptake in rubber seedlings (Kanyasone, 2009).

Mimosa invisa, an annual leguminous plant that is easier to grow and manage than the tree *M. denticulata*, is already used as a green manure and cover crop by upland farmers in northern Thailand (Rerkasem and Pinedo-Vasquez, 2007). The possibility of using *M. invisa* in the production of AMF inoculum should be further investigated.

Conclusion

From this study, the following conclusions are reached: (1) All improved and local cowpea lines growing in the shifting cultivation system had sufficient P supply and highly associated with AMF; (2) Arbuscular mycorrhizal fungi from farmer's shifting cultivation fields have the potential to directly benefit cowpea growing in acid soils that is low in available P; (3) The AMF enhanced cowpea growth in acidic soil by improving P uptake efficiency of cowpea roots.

REFERENCES

Adjei-Nsiah S, Kuyper TW, Leeuwis C, Abekoe MK, Cobbinah J, Sakyi-Dawson O, Giller KE (2007). Farmers' agronomic and social evaluation of productivity, yield and N_2-fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. Nutr. Cycl. Agroecosyst. 80:199-209.

Arshad MA, Gill KS (1996). Field pea response to liming of an acid soil under two tillage systems. Can. J. Soil Sci. 76: 549-555.

Bolt GH (1979). Soil chemistry. Elsevier Scientific Publishing Company. Amsterdam, The Netherlands.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N (1996). Working with Mycorrhizas in Forest and Agriculture. The Australian Centre for

International Agricultural Research (ACAIR) Monograph No. 32, Canberra, Australia.
Craswell ET, Pushparajah E (1989). Management of acid soils in the humid tropics of Asia. ACIAR Monograph No. 13 (IBSRAM Monograph No. 1), Roden Print, Canberra (A.C.T.), Australia.
Dakora FD, Aboyinga RA, Mahama Y, Apaseku J (1987). Assessment of N_2-fixation in groundnut (*Arachis hypogaea*) and cowpea (*Vigna unguiculata* L. Walp) and their relative N contribution to a suceeding maize crop in Northern Ghana. MIRCEN J. 3: 389-399.
Edwards D, Kang B, Danso K (1981). Differential response of six cowpea (*Vigna unguiculata* (L.) Walp) cultivars to liming in an ultisol. Plant Soil, 59: 61-73.
Hafner H, Ndunguru BJ, Bationo A, Marschner H (1992). Effect of nitrogen, phosphorus and molybdenum application on growth and symbiotic N_2-fixation of groundnut in an acid sandy soil in Niger. Fert. Res. 31: 69-77.
Harter RD (2002). Acid Soils of the tropics [Internet]. c2002-2011. Nort Fort Myers (FL) 33917 USA: ECHO Technical Note; [cited 2011 Feb 13]. Available from: http://people.umass.edu/psoil370/Syllabus-files/Acid_Soils_of_the_Tropics.pdf
Haynes RJ, Ludecke TE (1981). Effect of lime and phosphorus applications on concentrations of available nutrients and Mn uptake by two pasture. Plant Soil, 62: 117-128.
International Plant Nutrition Institute (2009). Managing acid upland soils in Southeast Asia [Internet]. c2009-2011. Norcross (GA) 30092 USA: International Plant Nutrition Institude; [cited 2011 Jan 31]. Available from: http://seap.ipni.net/articles/SEAP0031-EN
Ikombo BM (1991). Phosphorus Nutrition of Tropical Crop Legumes [PhD Thesis]. Brisbane (QLD): University of Queensland.
Kanyasone V (2009). Arbuscular Mycorrhizal Fungi (AMF) for Rubber Seedlings and Agroforestry in Northern Lao P.D.R. [MS Thesis]. Chiang Mai: Chiang Mai University.
Kittiworawat S, Youpensuk S, Rerkasem B (2010). Diversity of arbuscular mycorrhizal fungi in *Mimosa invisa* and effect of the soil pH on the symbiosis. Chiang Mai J. Sci. 37: 517-527.
Maddox JJ, Soileau JM (1991). Effects of phosphate fertilization, lime amendments and inoculation withVA-mycorrhizal fungi on soybeans in an acid soil. Plant Soil, 134: 83-93.
Marschner H (1995). Mineral Nutrition of Higher Plants. 2nd ed. Academic Press, London, UK.
Murphy V, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta, 27: 31-36.
Othman WM Wan, Lie TA, Mannetje L't, Wassink GY (1991). Low Level Phosphorus Supply Affecting Nodulation, N_2 Fixation and Growth of Cowpea (*Vigna unguiculata* L. Walp). Plant Soil, 135: 67-74.
Parkpian P, Pongsakul P, Sangtong P (1991). Characteristics of acid soils in Thailand: A review. In Plant-Soil Interaction at Low pH. Eds. Wright R J, Baligar V C, Murrmann R P,. Proceeding of the Second International Symposium on Plant-Soil Interactions at Low pH; Beckley, West Virginia, USA, pp. 397-405.
Peoples MB, Brockwell J, Herridge DF, Rochester IJ, Alves BJR, Urquiaga S, Boddey RM, Dakora FD, Bhattarai S, Maskey SL, Sampet C, Rerkasem B, Khan DF, Hauggaard-Nielsen H, Jensen ES (2009). The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis, 48: 1-17.
Piedra R, Munns DN (1990). Soil Acidity and Liming Effects on Stand, Nodulation, and Yield of Common Bean. Agron. J. 82: 749-754.
Rajapakse S, Zuberer DA, Miller JC (1989). Influence of phosphorus level on VA Mycorrhizal colonization and growth of cowpea cultivars. Plant Soil, 114: 45-52.
Rerkasem K, Pinedo-Vasquez M (2007). Diversity in Smallholder Systems and Responses to Environmental and Economic Changes. in Managing Biodiversity in Agricultural Ecosystems, Eds. Jarvis D, Padoch C, Cooper H D, pp. 362-381. Columbia University Press, New York, USA.
Steele WM, Allen DJ, Summerfield RJ (1985). Cowpea (*Vigna unguiculata* (L.) Walp.). In Grain Legume Crops, Eds. Summerfield RJ, Roberts EH, pp. 520-583. William Collins Sons & Co, London, U.K.
von Uexk˜ull HR, Mutert E (1995). Global extent, development and economic impact of acid soils. In Plant-Soil Interactions at Low pH: Principles and Management, Eds. Date RA, Grundon NJ, Raymet GE, Probert ME. Dordrecht, Kluwer Academic, The Netherlands, pp. 5-19.
Wongmo J (2008). Influences of Arbuscular Mycorrhizal Fungi on Different Food Crops [Ph.D. Thesis]. Chiamg Mai; Chiang Mai University.
Wongmo J, Dell B, Lumyong S, Rerkasem B (2008). Shifting cultivation system and crop symbiosis with arbuscular mycorrhizal fungi. CMU. J. Nat. Sci. 7: 269-277.
Yimyam M (2006). Arbuscular Mycorhizal Fungi on Growth of Arabica Coffee Seedling. Highland Research and Training Center, Faculty of Agriculture, Chiang Mai University, 20pp.
Yimyam N, Rerkasem K, Rerkasem B (2003). Fallow enrichment with pada (*Macaranga denticulata* (Bl.) Muell. Arg.) trees in rotational shifting cultivation in Northern Thailand. Agroforest. Syst. 57: 79-86.
Yimyam N, Youpensuk S, Wongmo J, Kongpan A, Rerkasem B, Rerkasem K (2008). Arbuscular mycorrhizal fungi, an underground resource for sustainable upland agriculture. Biodiversity, 9: 61-63.
Youpensuk S, Dell B, Lumyong S, Rerkasem B (2004). Arbuscular mycorrhizal fungi from the rhizosphere of a fallow enriching tree, *Macaranga denticulata Muell*. Arg. and their effect on the host plant. Agroforest. Syst. 60: 239-246.
Youpensuk S, Yimyam N, Lumyong S, Rerkasem B, Dell B (2005a). Arbuscular mycorrhizal fungi associated with upland rice in a rotational shifting cultivation system. Int. Rice Res. Notes, 30: 22-23
Youpensuk S, Rerkasem B, Dell B, Lumyong S (2005b). Effects of arbuscular mycorrhizal fungi on a fallow enriching tree (*Macaranga denticulata*). Fungal Divers. 18: 189-199.
Youpensuk S, Lordkaew S, Rerkasemc B (2006). Comparing the Effect of Arbuscular Mycorrhizal Fungi on Upland Rice and *Macaranga denticulata* in Soil with Different Levels of Acidity. Sci. Asia, 32: 121-126.
Zahran HH (1999). *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. R. 63: 968-989.