CHAPTER 3

ARBUSCULAR MYCORRHIZAL STATUS OF LEGUMES IN A SHIFTING CULTIVATION SYSTEM IN NORTHERN THAILAND

3.1 Introduction

In the highlands of northern Thailand, shifting cultivation is the main form of agriculture. In the old days sustainable shifting cultivation was achieved by rotation with 9-14 years of fallow between cropping years (Rerkasem and Rerkasem, 1994). In recent years the rotation cycle has been much reduced, because of population pressure and implementation of the Thai government's conservation policy. However in Huai Teecha village, Sob Mei district, Mae Hong Son province in, the rotation cycle has been reduced to just 7 years (Yimyam *et al*., 2003). On soil that is mainly acidic and deficient in phosphorus (P) for plant growth, local farmers have a special rotation system to regenerate the land. The local knowledge that high density of a fallow enriching tree, *Macaranga denticulat*a is associated with high rice yield has been definitively verified (Yimyam *et al*, 2003). This tree is highly dependent on arbuscular mycorrhizal fungi (AMF); in the field its roots are well colonized by the AMF and there is high density of AMF spore in the rhizosphere (Youpensuk *et al*. 2004). The local population of AM fungi associated with *M. denticulata* at Huai Teecha is especially diverse and abundant (Youpensuk *et al*., 2004) and are effective in improving the growth of many crop species, including rubber (Kanyasone, 2009), coffee (Yimyam, 2006) and tangerine (Youpensuk *et al*., 2008). Food crops of shifting cultivation at Huai Teecha, including upland rice, job's tears and sorghum have also been shown to benefit from association with the AMF (Wongmo, 2008).

Since many legumes are also routinely grown in shifting cultivation fields, this study aimed to explore the extent of AMF association in grain legumes within the cropping system at Huai Teecha. The specific objectives were to determine the extent of colonization of AMF in legume roots and the abundance of AMF spore in soil in the root zone of legume and to determine effects of legume genotype on mycorrhizal colonization.

3.2 Materials and methods

Experiment 3.2.1 Mycorrhizal status of local legumes in shifting cultivation system

The field survey examined AMF infection in 3 grain legume species: cowpea (*Vigna unguiculata* L. Walp.), yard long bean (*Vigna unguiculata* ssp. *Sesquipedalis* (L.) Verdc.) and winged bean (*Psophocarpus tetragonolobus* (Linn.) DC.) in Huai Teecha village during the middle of the wet season in 2005. Fields of four farmers (Mr Takae, Mr Kayo, Mr Da and Mr Ducare) were surveyed, one field per farmer in upper, mid and lower slope positions. Roots of 3 replicated plants per species per field were excavated for measuring level of infection. Two soil cores (4.5 cm diameter x 15 cm depth) were taken from the base of each plant and combined. These samples were used for soil analysis and spore extraction. One hole (50 x 50 cm wide and 60 cm deep) was excavated at each altitude in Kayo field and soil samples removed from the pit wall to measure spore number in the soil profile. Roots were carefully washed to free from the soil and cut to 1 cm root pieces. Then roots 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. The soil samples were air dried and 30 g of each was used for spore extraction and the remainder was passed through a 2 mm screen and soil pH (1:1, H_2O) and Bray II P determined. Spores were extracted by wet sieving and sucrose centrifugation (Brundrett *et al*. 1996). The extracted spores were transferred to filter paper and counted under a stereo microscope.

Experiment 3.2.2 Evaluating arbuscualr mycorhizal fungi stutus in different cowpea cultivars in a shifting cultivation system

Experimental site selection

Because the experiment had to be conducted in low P acid soil, the low P acid soil in Huai Teecha must be located before commencing the experiment. To find the suitable

experimental site 6 farmer fields (Tongdee, Noppon, Kayo, Por, Lar and Luyo) in Huai Teecha village were surveyed in late April 2008 (before growing season). Soil of each field was randomly corrected and primary check by pH test kid (produced by Central laboratory Faculty of Agriculture Chiang Mai University). Then the acid soil areas (pH< 5.5) were marked and sampled (Figure 3.1) for soil analysis in laboratory (pH and available P) and counting AMF spore (by the method described previously).

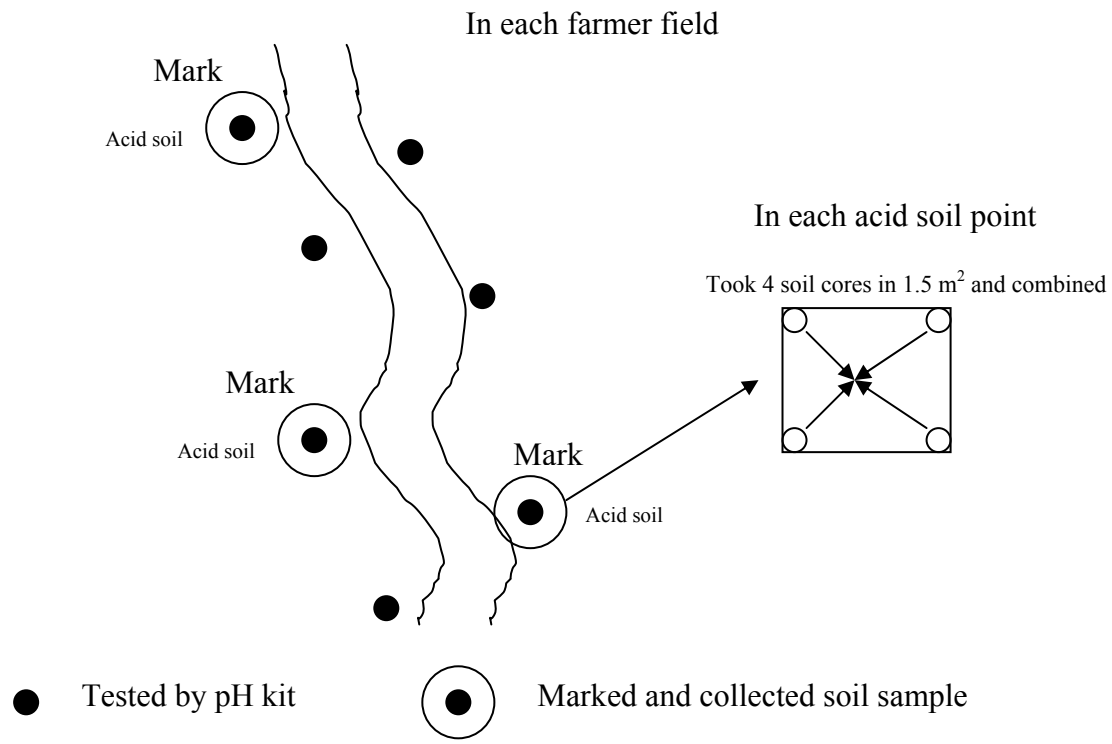

Figure 3.1 Method of soil sample collection. In each farmer field soil pH was measured by pH testing kit (provided by central laboratory of Faculty of Agriculture Chiang Mai University) along the track. The acid soil points (pH ≤ 6) were marked and a soil sample taken from each point. In each point 4 soil cores were taken and combined as a soil sample.

The fields that have at least 3 points of acid soil and low soil P concentration were chosen to set the experiment.

Testing the effect of cowpea genotype on mycorrhizal symbiosis

Three farmer's fields including Tongdee, Kayo and Luyo field were chosen for experiment locations. Four cowpea lines including ITD-1131, cv. Ubon Ratchathani, IT90K-227-2 and a local variety collecting from Tongdee field were grown with 3 replications in each field. In each field the experiment was arranged in randomize complete block Design (RCB) with 3 replications. A 50 cm^2 plot was an experimental unit and 8 plants were sown in each plot. At 50 days after sowing root of 2 plants in each plot was excavated to measure AMF root colonization and spore density by method as describe before. Youngest Full Expended Leave (YFEL) of 4 plants in each plot was collected to measure P concentration by Molybdovanadate-Phosphoric Acid method (Murphy and Riley, 1962). Two soil cores (4.5 cm diameter and 15 cm deep) from base of 2 plants were collected in each plot and combined to measure pH (1:1, H_2O) and soil P concentration (Bray II) and amount of AMF spore.

Data was analyzed using analysis of variance and difference amount treatments were compared using least significant different (LSD) value ($P \leq 0.05$). The data in percentage was transformed by arcsine before analysis. Correlation coefficient was calculated to determine relationship between factors.

3.3 Result

3.3.1 Mycorrhiza status of local legumes in shifting cultivation system

Roots of the legumes in Huai Teecha village were colonized by AMF ranging from 39 to 98%. The root colonization percentage was not different between legume species in each farmer's field (Figure 3.2).

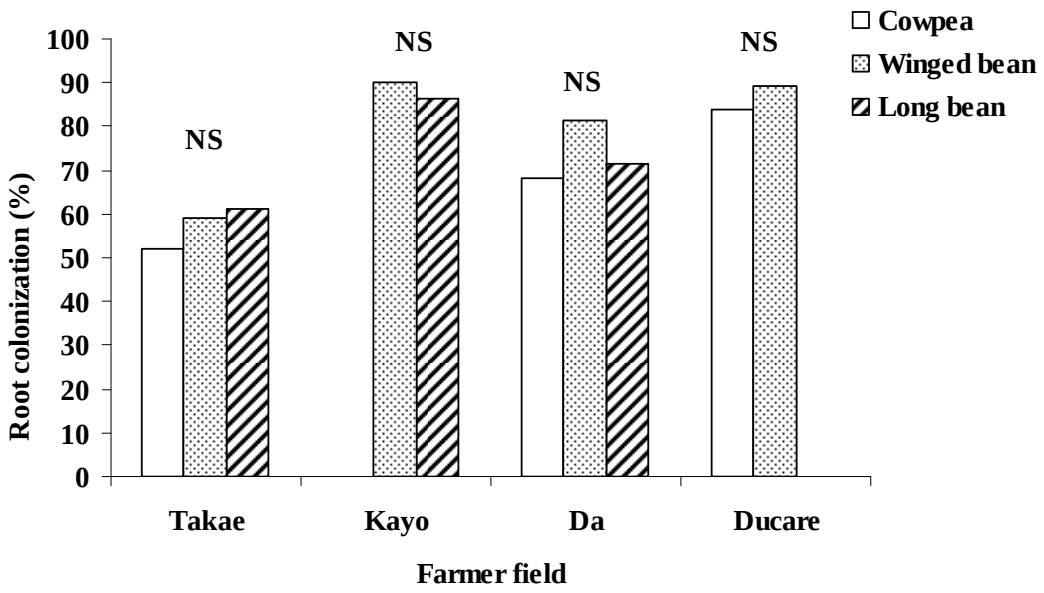

Figure 3.2 Abuscular mycorrhiza fungi colonization in roots of 3 legumes in 4 farmer's fields at Huai Teecha. Values are means from 3 replications. NS = non-significant by t-test ($P < 0.05$).

The significant negative correlation between soil pH and root colonization was found (r = -0.461). Root colonization of legumes was higher when soil was more acidic (Table 3.3). Soil P was a major factor to determine root colonization of legumes in this village that was shown by highly significant negative correlation between soil P concentration and root colonization percentage (r = -0.752). Root colonization became higher when soil P became lower (Table 3.1). There was no correlation between root colonization and spore density in root zone (Table 3.1). Spore density in root zone of legumes did not relate with soil pH or soil P (Table 3.1).

Table 3.1 Correlation coefficient between root colonization, soil pH and soil P and spore density in root zone soil.

	Root colonization	Soil pH	Soil P
Root colonization	.		
Soil pH	-0.469*		
Soil P	-0.752**	0.354	
Spore density	0.061	-0.049	0.107

* = significant different at $P < 0.05$, ** = significant different *at* $P < 0.01$

The study of spore distribution along soil profile was conducted in Kayo's field only (Table 3.2). The AMF spores were found throughout soil profile from top soil to 50 cm deep. The distribution of spore was different between locations. In upper slope pit, most of the spores were at 5-10 cm deep (21.67 spores/ g soil), and very few spores were found at 30-40 cm (0.04 spores/ g; Table 3.2). In the mid-slope pit the highest spore density was found in 0-5 cm deep level (0.6 spores/ g) and the lowest was found

in 20-30 cm deep (0.23 spores/ g; Table 3.2). In the lower slope pit the highest spore density was found in 0-5 cm deep level (1.84 spores/ g) and the lowest was found in the bottom level of the pit (40-50 cm deep, 0.4 spores/ g).

Table 3.2 Distribution of AMF spore in the soil profile at 3 locations in Kayo field in Haui Teecha.

Soil deep	Spore Number /g dry soil			mean	SE
	Pit 1 (Upper slope)	Pit 2 (Middle slope)	Pit 3 (Lower slope)		
0-5 cm	1.01	0.60	1.84	1.15	0.36
5-10 cm	21.67	0.43	0.30	7.47	7.10
10-15 cm	0.33	0.25	0.48	0.35	0.07
15-20 cm	1.54	0.39	0.67	0.87	0.35
20-30 cm	0.50	0.23	0.15	0.29	0.11
30-40 cm	0.04	0.46	0.15	0.22	0.13
40-50 cm	0.18	0.41	0.04	0.21	0.11
CV (%)	2.21	0.32	1.21		

3.3.2 Evaluating arbuscualr mycorhizal fungi status in different cowpea cultivars in a shifting cultivation system

From the field survey in Huai Teecha soil pH , soil P and AM fungi spore density in soil, varied between farmer fields. Soils in all farmer fields were acidic. Soil pH ranged between 5.08 and 5.65. The most acidic soil was found in Loyo field (pH 5.08, Figure 3.3). Soil in all farmer fields contained low P (ranging from 0.74 to 6.25). The low soil P was found in Luyo field (Figure 3.4). Spore density between farmer fields varied from 2.83 to 6.94 spores/g and the highest spore density was found in Tongdee field (Figure 3.5). Therefore Tongdee, Kayo and Luyo field were chosen to represent rich AM fungi spore, extremely low P and most acidic soil respectively for conducting the experiment.

Table 3.3 Soil pH, soil P concentration and spore density in soil of 6 farmer's field the values are mean from 3 replications (± standard error)

Famer	Soil pH	Soil P concentration (mg P/kg)	AMF spore density (spores/g soil)
Tongdee	5.65 ± 0.06	2.20 ± 1.03	6.94 ± 1.79
Noppond	5.38 ± 0.05	6.25 ± 3.71	3.08 ± 0.11
Kayo	5.13 ± 0.08	0.74 ± 0.06	3.01 ± 1.25
Por	5.45 ± 0.11	6.12 ± 3.54	3.37 ± 0.78
Lar	5.30 ± 0.03	2.90 ± 0.68	4.31 ± 0.44
Luyo	5.08 ± 0.05	2.79 ± 0.42	2.83 ± 0.18

Root colonization P concentration in YFEL of and spore density in root zone soil of 4 cowpea cultivars were not different in each farmer field (Figure 3.3 to 3.5). The root colonization was high ranging between 73 to 90% (Figure 3.3). Phosphorus concentration in YFEL ranged between 0.23 and 0.338% (figure 3.4).

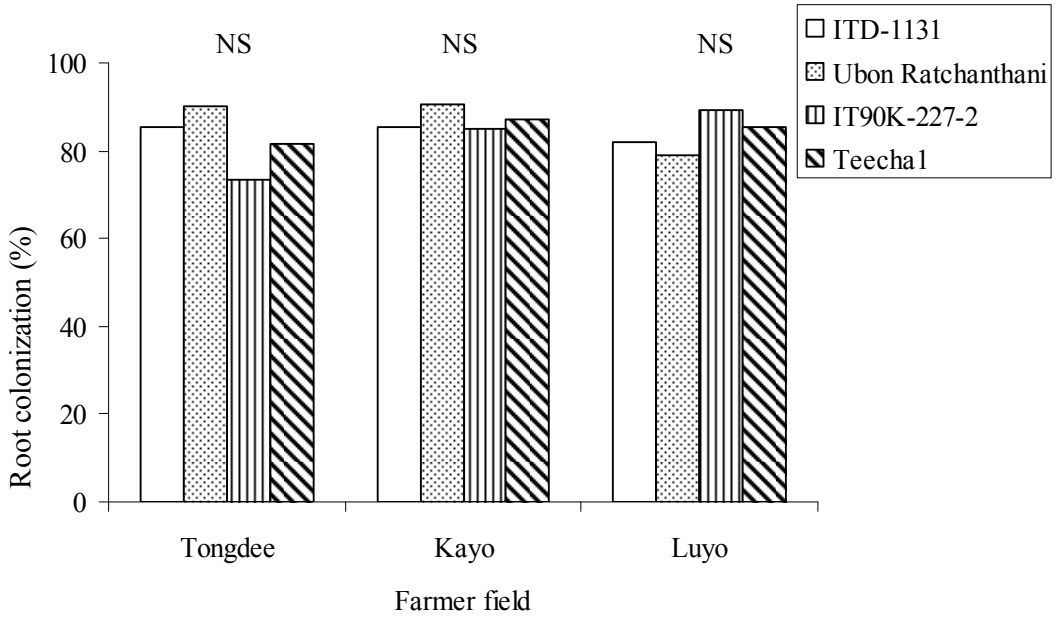

Figure 3.3 Arbuscular mycorrhiza fungi root colonization in 4 cowpea lines in 3 farmer fields. (Data was transformed by arsine before analysis of variance)
NS = non-significant difference

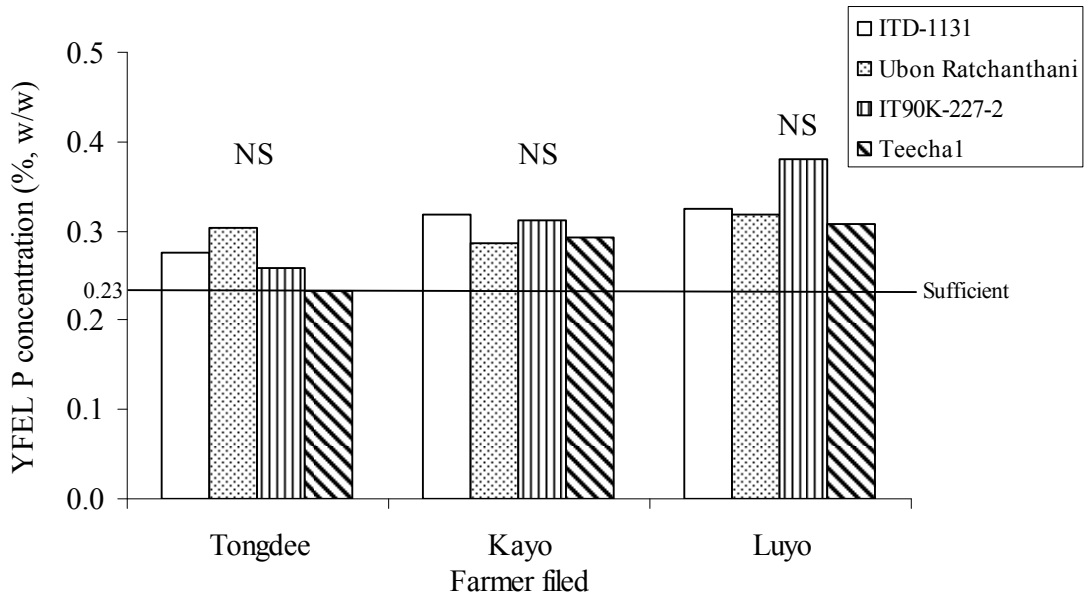

Figure 3.4 Phosphorus concentration in youngest full expended leaf (YFEL) of 4 cowpea lines (one local variety plus 3 introduced improved lines) in 3 farmer's fields. Sufficient = sufficient P level (90% maximum yield; Ikombo, 1991)

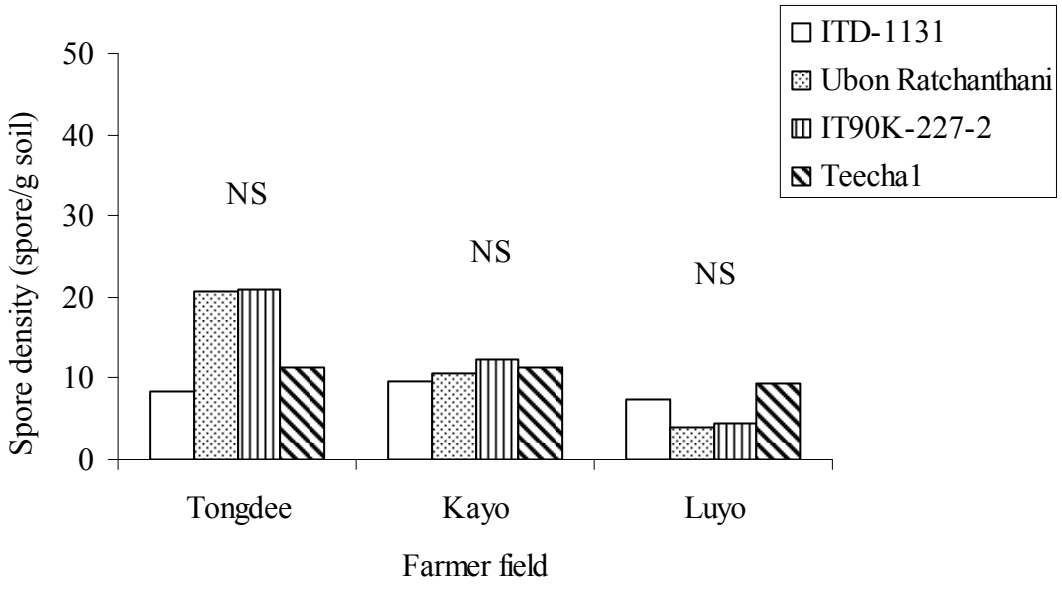

Figure 3.5 Spore density in root zone soil of 4 cowpea lines in 3 farmer fields. NS = non-significant difference

The negative correlation between soil P and root colonization was found. Low soil P enhanced root colonization (Table 3.4). Root colonization was not related with spore density in root zone soil, soil pH and P in YFEL. Spore density was not affected by any soil pH and soil P (Table 3.4).

Table 3.4 Correlation coefficient between root colonization, P concentration in YFEL, Soil P and soil pH

	Root colonization	YFEL P concentration	Soil P concentration	Soil pH
P concentration in YFEL	0.174			
Soil P concentration	-0.400*	-0.070		
Soil pH	-0.162	-0.241	-0.006	
Spore density	-0.186	-0.198	0.129	0.320

* = significant different at $P < 0.05$

3.4 Discussion

The significant inverse correlation between AMF root colonization and soil P or soil pH indicated that there was greater symbiotic development between legumes and AMF symbiosis when soil is more acidic or has less available P. The lessening of AMF root colonization with increasing soil P found here in farmers' fields agrees with general observation that plant's dependence on AMF symbiosis declines with increasing soil P (Peng *et al*., 1993; Valentine *et al.*, 2001). The result supported an idea that legumes growing on acidic low P soil in this village could benefit from association with the AMF. The absence of correlation between root colonization and soil pH in experiment 3.2.2 may be related to the narrower range of soil pH in this data set. The acid soil areas were chosen to be used as experimental sites. This makes the pH ranging between 5.1 and 5.8 while soil pH ranged was between 5.5 and 7.4 in survey work (experiment 3.2.1). The AMF heavily colonized roots of all planted

legumes species. They also colonized roots of improved cowpea lines just as well as the local variety. The P status of all cowpea lines in experiment 3.2.3 was in sufficient range (Figure 3.4). That means local and improved lines can stand without P deficiency in low P acid soil of Teecha.

Legumes in Haui Teecha village highly associated with the endogenous AMF especially when the face of low P acid soil. There was no evidence introduced that improved varieties of cowpea was different from the local cowpea in root infection and plant P status as indicated by P concentration of the YFEL. All improved and local cowpea lines growing in the low P acid soil in Haui Teecha was not stressed by P deficiency. The two experiments in this chapter have shown that AMF could also directly benefit legumes growing on acidic low P soil in the highlands.