CHAPTER 3

MATERIALS AND METHODS

3.1 Experiment 1: Effects of cassava stake priming with complete nutrient solution on growth and yield

This experiment was conducted at the Mae Hia Research Station and Training Center, Chiang Mai University, Thailand in September 2010 to August 2011. The location had annual rainfall of 1 685.4 mm and average daily temperature of 25.52°C (Figures 1 and 2). The soil of the site was characterized before planting cassava (Table 2).

The treatments were arranged in a factorial combination in a randomized complete block design (RCBD), with four replications. The combinations were stake priming (two levels: priming and nil-priming) and cassava varieties (Rayong 5, Rayong 7, Rayong 9, Rayong 72, and Kasetsart [KU] 50). Stakes of the five cassava varieties were obtained from the same source − Chiang Mai Field Crop Research Center, Sansai, Chiang Mai. Concentrations of N, P and K for the stakes were determined prior to commencement of the experiment (Table 3). Stakes of 20-cm length were primed by submerging in a complete nutrient solution (Table 4) for two hours and then they were planted vertically with 1 x 1 metre spacing in 4 x 5 metre experimental units. Weed control was done by hand. The experiment was completed at 12 months after planting.

Six plants from the internal area of each plot were harvested at 12 months after planting. The number of roots, aboveground biomass, fresh and dry root weight, starch content and starch yield were measured. Leaf and stem samples were oven-dried at 75°C for 48 hours, and root samples were chopped and oven-dried at 75°C for 72 hours, before grinding separately. Stems, leaves and roots were analyzed separately to determine N, P and K concentrations and combined for whole plant

nutrient content; 2 g of ground samples were dry-ashed at $500°C$ for eight hours and dissolved in 0.1 N HCL and then P was determined by using a colorimetric assay (the molybdovanado-phosphoric acid method) and a spectrophotometer (Murphy and Riley 1962); K was determined by an atomic absorption spectrophotometer. Determination of N concentration in each plant part used the Kjeldahl method. Starch content of fresh roots was estimated by determination of root specific gravity (Wholey and Booth 1979), the method used commercially to determine starch content and price in selling and buying of cassava roots). The starch content of fresh roots was calculated with the following formula:

Starch content (%) = 210.8 specific gravity – 213.4;

When **specific gravity = weight sample in air/(weight sample in air – weight sample in water)**.

Table 2 Chemical and physical characteristics of the soil used for experiment 1.

Soil characteristics	
Texture	Sandy loam
pH (in water, 1:1)	5.8
N (%)	0.7
P (μg g^{-1} Bray II)	15.5
K (meq 100 g^{-1} by 1N NH_4-acetate)	0.2
Other characteristics	Lao humus, organic matter and poor fertility

Table 3 Nutritional characteristics of the stakes used for experiment 1 before priming.

Nutrient elements	Varieties				
	Rayong 5	**Rayong 7**	**Rayong 9**	**Rayong 72**	**KU 50**
	Concentration (g kg^{-1})				
N	4.5	4.2	3.6	4.9	4.8
P	2.6	3.0	2.8	3.1	1.0
K	5.6	6.2	4.6	4.9	3.9

Table 4 Elements and salts used in the complete nutrient solution.

Stock and solution	Elements	Concentration need (ppm)	Formula	Preparing of stock solution (g/L)	Priming solution (mL/10 L)
1	N	25 000	KNO_3	202.2	25
2	P	5 000	KH_2PO_4	136.09	25
3	K	1 250	K_2SO_4	87	25
4	Ca	5 000	$CaCl_2. 2H_2O$	294.1	25
5	Fe	250	Fe-citrate	6.7	25
6	Mg	1 250	$Mg_2SO_4. 7H_2O$	123.3	25
	Mn	5	$MnSO_4. H_2O$	0.338	
7	Zn	2.5	$ZnSO_4. H_2O$	0.288	25
8	Cu	0.1	$CuSO_4. 5H_2O$	0.1	25
	Co	0.5	$CoSO_4. 7H_2O$	0.056	
	Mo	0.5	$Na_2MoO_4. 2H_2O$	0.048	
9	B	50	H_3BO_3	0.247	250

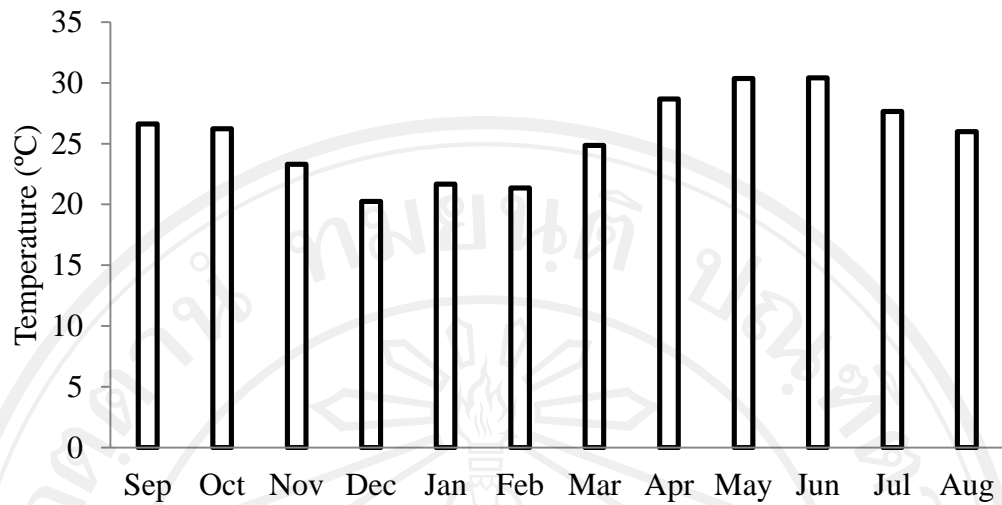**Sources:** Broughton and Dilworth (1971).

Figure 1 Mean temperature at Mea Hai Research Station and Training Center during the period of the trial, 2009−2010.

Source: Division of Soil Science, Department of Plant Sciences and Natural Resources, Faculty of Agriculture, Chiang Mai University, 2011.

Figure 2 Rainfall at Mae Hia Research Station and Training Center during the period of the trial, 2009−2010.

Source: Division of Soil Science, Department of Plant Science and Natural Resources, Faculty of Agriculture, Chiang Mai University, 2011.

3.2 Experiment 2: The responses to stake priming in KU 50 from different sources in Lao PDR on growth and yield

This experiment was conducted at the Rice and Crash Crops Research Center (RCCRC), National Agriculture and Forestry Research Institute (NAFRI), Vientiane, Lao PDR (N 18° 08' 44", E 102° 44' 05", 168 metres above sea level) in May 2010 to March 2011. The soil of the site was characterized before planting (Table 5).

The treatments were arranged in a factorial design of two combinations arranged in a RCBD, with three replications. The combinations were two levels of stake priming with complete nutrient solution for two hours before planting (priming and nil-priming) and four stakes of the cassava variety KU 50, a popular variety in Lao PDR.

The stakes of KU 50 were taken from four different sources in Lao PDR (Figure 3) where the mother plants were grown:

(i)   Naphok, Xaithany District (Naphok) (N 18° 08' 44", E 102° 44' 05", 168 metres above sea level), Vientiane capital; located in the middle region where cassava is mostly grown on lowlands for starch factories.

(ii) Peak District (Peak) (N 19° 20' 35", E 103° 09' 10", 1 102 metres above sea level).

(iii) Phaxay District (Phaxay) (N 19° 17' 44", E 103° 04' 57", 1 121 metres above sea level), Xiengkhuoang Province; located in northern Lao PDR where cassava is mostly grown on sloping land with high soil erosion, for household consumption and animal feed.

(iv) Xay District (Xay) (N 20° 41' 57", E 101° 59' 29", 640 metres above sea level), Oudomxay Province; located in northern Lao PDR.

Stake concentrations of N, P and K were determined prior to commencement of the experiment (Table 6). Stake priming involved submerging the stakes in a complete nutrient solution (modified from Broughton and Dilworth [1971]) for two hours before planting. The nutrient solution used for the experiment was the same as

experiment 1 (Table 4). Stakes of 20-cm length were planted vertically in 6 x 5 metre experimental units with 1 x 1 metre spacing. Weed control was done by hand. The experiment had two harvests (at four and eight months after planting).

Three plants were harvested at four months and six plants at eight months after planting from the internal area of each plot. The number of roots, aboveground biomass, fresh and dry root weight, starch content and starch yield were measured. Starch content was measured and calculated using the same formula as experiment 1.
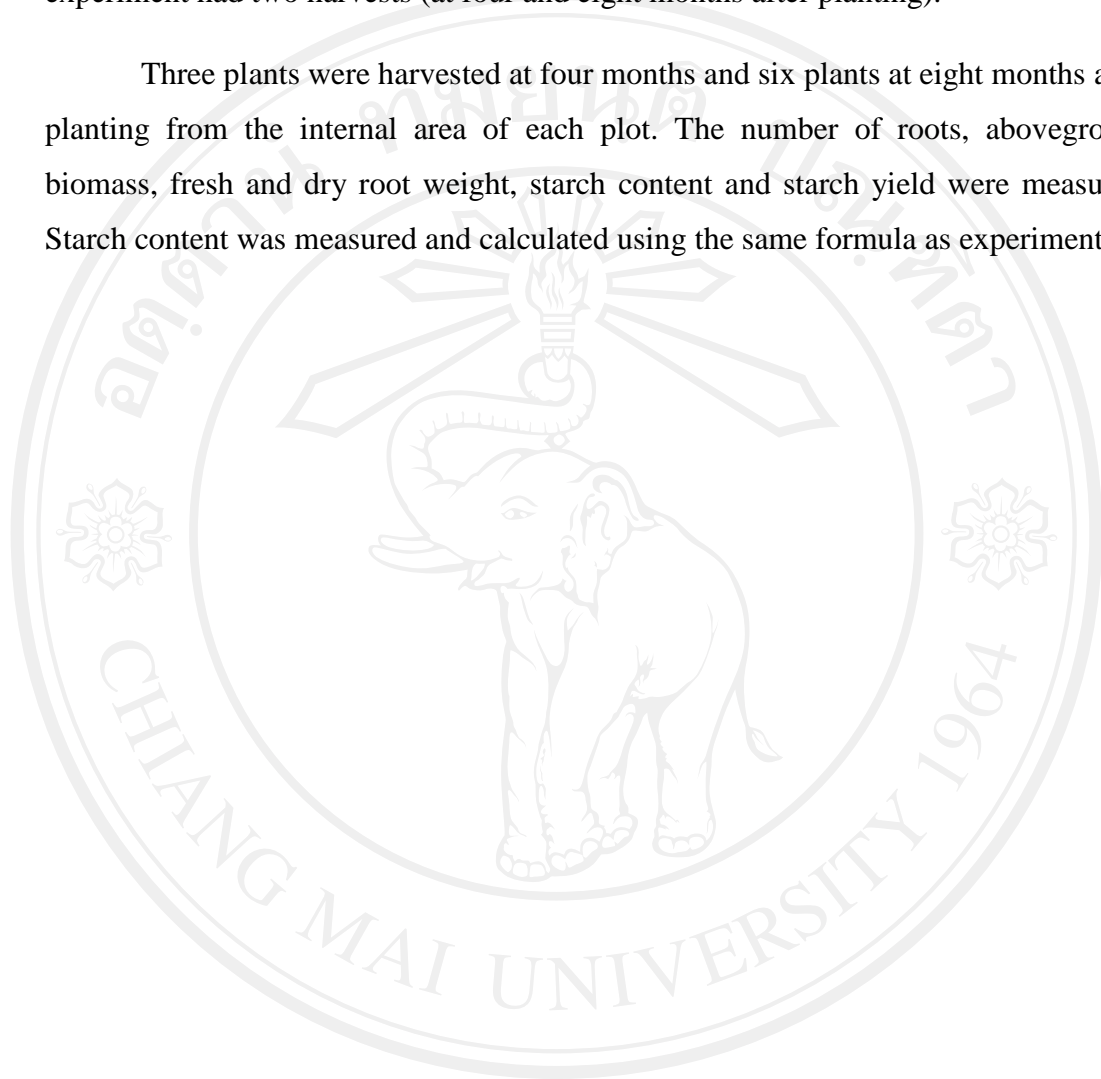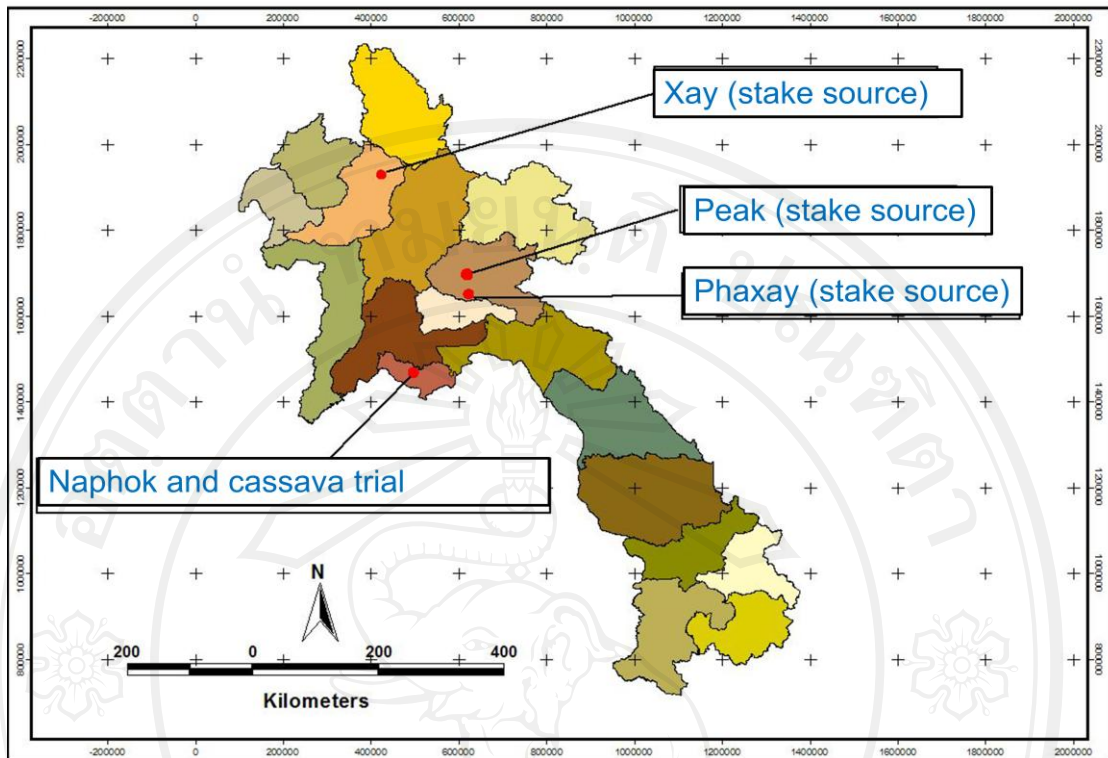
Figure 3 Location of the plot site and stake source for experiment 2 in Lao PDR.

Table 5 Chemical and physical characteristics of the soil used for experiment 2.

Soil characteristics	
Texture	Loam
pH (in water, 1:1)	4.5
Organic matter (%)	1.8
N (%)	0.1
P ($\mu g\ g^{-1}$ by Bray II)	6.3
K (meq 100 g^{-1} by 1N NH_4-acetate)	0.1
Zn ($\mu g\ g^{-1}$ by DTPA)	0.6

Table 6 Nutritional characteristics of the stakes used for experiment 2 before priming.

Nutrient element	Stake sources			
	Naphok	Peak	Phaxay	Xay
	Concentration (g kg^{-1})			
N	7.5	7.1	3.8	7.3
P	0.7	0.6	0.9	1.0
K	7.2	1.6	4.2	5.7

3.3 Experiment 3: Response to stake priming in cassava varieties from different sources in Thailand on growth, yield and nutrient accumulation

This experiment was conducted at the Mae Hia Field Station of Chiang Mai University, Thailand from June 2010 to April 2011. The location had annual rainfall of 1 746 mm (Figure 4) and average daily temperature of 25°C (Figure 5). The soil of the site was characterized before planting (Table 7).

The treatments were a factorial combination of three factors arranged in an RCBD with four replications. The factors were stake priming (two levels: priming and nil-priming), cassava varieties (two very popular varieties in Southeast Asia, KU 50 and Rayong 72) and stake sources (three locations where the mother plants were grown: Mukdahan and Mahasarakham in northeast Thailand, the country's main cassava-growing region and from Chiang Mai in the north) (Figure 6). Stake concentration of N, P and K was determined prior to commencement of the experiment (Table 8).

Stake priming involved submerging the stakes in a complete nutrient solution (modified from Broughton and Dilworth, 1971) for two hours before planting. The nutrient solution used for the experiment was the same as experiment 1 (Table 4). Stakes of 20-cm length were planted vertically in 5 x 6 metre experimental units with 1 x 1 metre spacing. Weed control was done by hand.

Three and six plants from the internal area of each plot were harvested at four and eight months after planting. At each harvest, the number of roots, aboveground biomass, fresh and dry root weight, starch content and starch yield were measured. Leaf, stem and root sample preparation for N, P and K and determining starch content also used the same formula as experiment 1.

Table 7 Chemical and physical characteristic of the soil used for experiment 2.

Soil characteristics	
Texture	Sandy loam
pH (in water, 1:1)	6.1
Organic matter (%)	1.3
N (%)	0.1
P (μg g^{-1} by Bray II)	6.5
K (meq 100 g^{-1} by 1N NH$_4$-acetate)	0.1
Zn (μg g^{-1} by DTPA)	1.2

Table 8 Nutritional characteristics of the stakes used for experiment2 before priming.

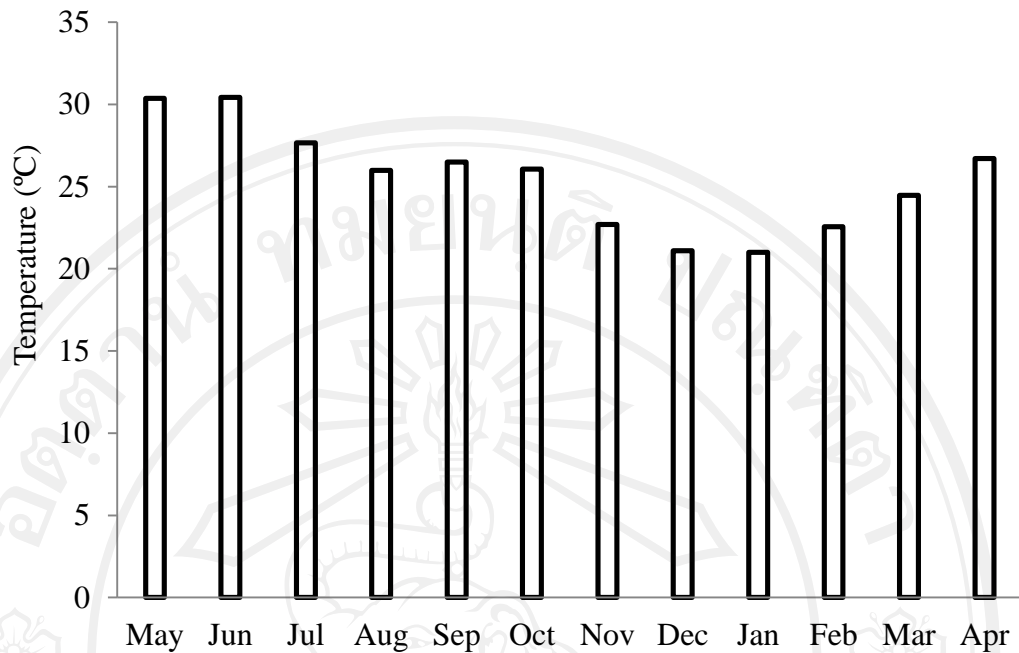| Variety	Stake sources								
	Chiang Mai			Mahasarakham			Mukdahan		
	N	P	K	N	P	K	N	P	K
	Nutrient concentration (g kg^{-1})								
Rayong 72	6.2	3.3	7.2	4.0	3.2	0.9	5.3	3.0	3.7
KU 50	4.9	3.1	5.1	5.0	2.3	1.5	5.9	1.9	6.6

Figure 4 Mean temperature at Mea Hai Research Station and Training Center during the period of the trial, 2010−2011.

Source: Division of Soil Science, Department of Plant Sciences and Natural Resources, Faculty of Agriculture, Chiang Mai University, 2011.
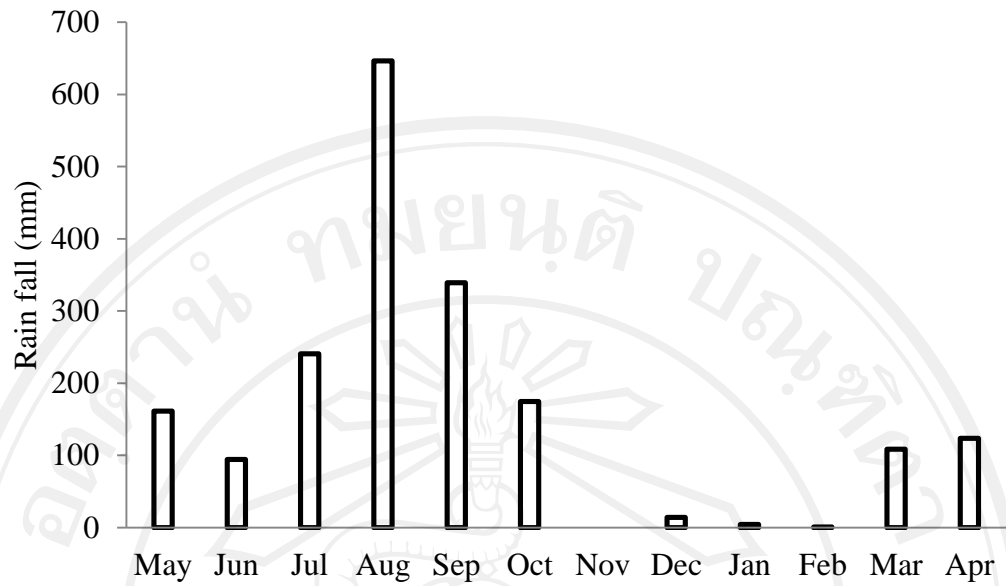
Figure 5 Rainfall at Mae Hia Research Station and Training Center during the period
of the trial, 2010−2011.

Source: Division of Soil Science, Department of Plant Sciences and Natural
Resources, Faculty of Agriculture, Chiang Mai University, 2011.
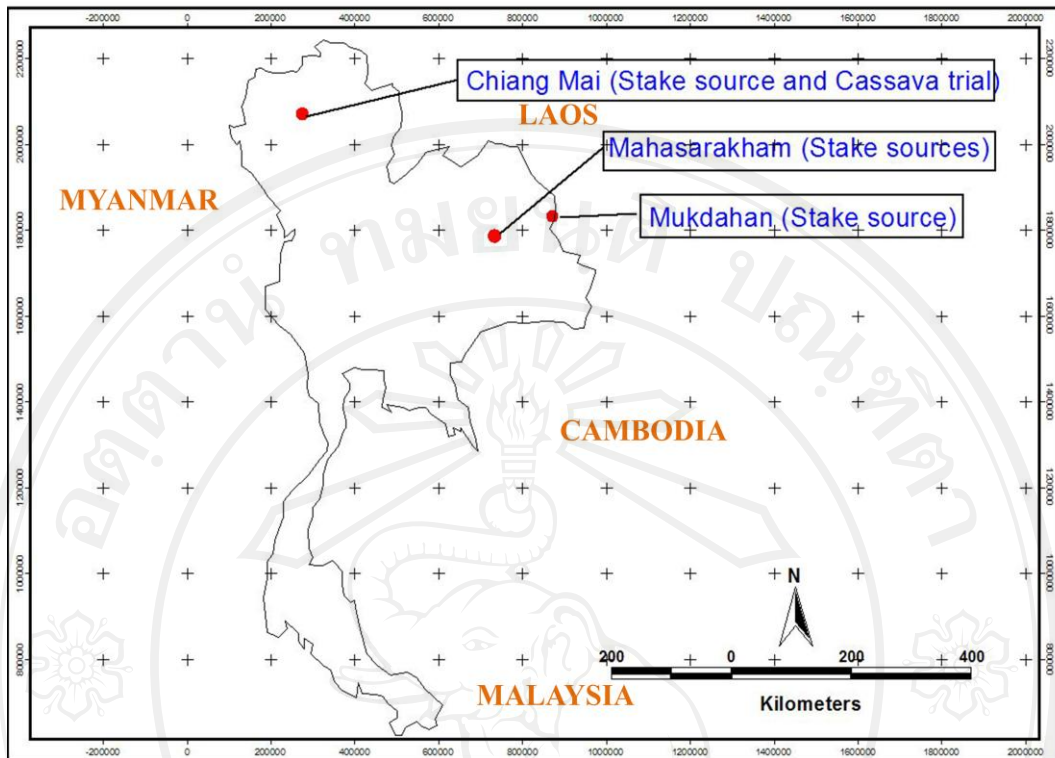
Figure 6 Location of the experimental site and sources of cassava stakes for experiment 3 in Thailand.

3.4 Experiment 4: Response to stake priming with nutrient solution on germination and early growth.

Cassava (*Manihot esculenta* Crantz), Rayong 72 was used in this study. The experiment was conducted in pots in the greenhouse at the CMUPN*lab*, Agronomy Division, Plant Science and Natural Resources Department, Chiang Mai University, Thailand from 1 to 30 June 2011. This experiment was conducted to evaluate the effect of stake priming on germination and early growth of cassava. The five stake treatments were: (1) priming in complete nutrient solution as in experiment 1 (Table 4), designated CN; (2) priming with 5 mM $CaCl_2$, designated Ca; (3) priming with 2.5 μM Zn_2SO_4, designated Zn; (4) priming in 50 μM H_3BO_3, designated B; and (5) nil-priming. The pots were arranged in an RCBD with four replications, giving a total of 40 plots, for two harvests at 15 and 30 days. Stake priming involved submerging the stakes in a CN, Ca, Zn and B nutrient solution (modified from Broughton and Dilworth 1997) for two hours and then leaving them to drain and dry overnight before planting.

Soil was collected from the same site of experiment 1. 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), with 9-kg samples placed in plastic pots. The mixture was adjusted from pH 5.8 to 7.5 with 2 g $CaCO_3$ kg^{-1} soil mixture (Figure 7). Three stakes of 20-cm length were planted vertically in each pot. The pots were watered daily. One set of pots was harvested at 15 and 30 days after planting. Germination was assessed daily to determine the germination rate until the crop completely germinated (15 days after planting). At each harvest, shoot and root number was counted, before oven-drying (at 75°C for 48 hours) for determination of dry weight.

Statistical analysis for all experiments

At the harvest, an analysis of variance (ANOVA) for all parameters measured was carried out to determine the treatment effects using commercial software (Statistix V.8, analytical Software, Inc.). Data were analyzed using ANOVA and treatment means were compared using least significant difference (LSD) at 5%.
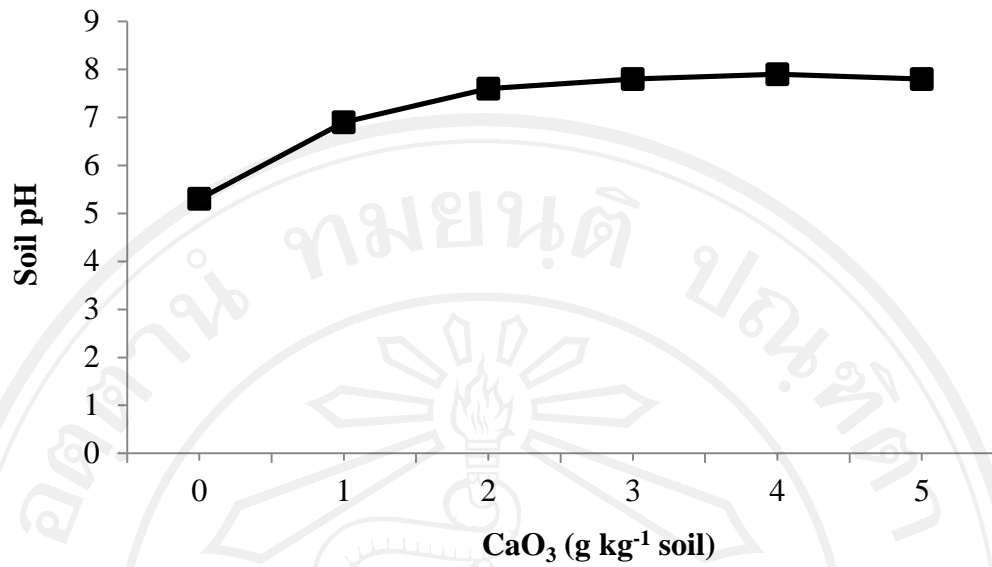
Figure 7 Adjustment of soil pH by CaO_3 before use for this experiment.

(a)

Figure 8 Measurement of starch content; (A) sample preparing (5 kg), (B) weight sample in water and (C) reading the sample weight.