CHAPTER III
MATERIALS AND METHODS

1. Location of Experimental Trial

The field experiment was conducted at Ban Thungpoe, Tambol Nonsa-at, Amphoe Sribunrueng, Nongboua Lamphu Province. The soil was Roi Et serie (Fine-loamy, mixed, isohyperthermic Aeric Paleaquults) (Soil Survey Division, 1972).

2. Experimental Period

The field experiment commenced on June 14, 1998 and ended on April 16, 1999. Analysis of soil and plant was undertaken in laboratory until July 2001. A total period of study was 2 years and 11 months.

3. Experimental Design

A factorial in Randomized Complete Block Design (FRCBD) of 2 factors involving with 12 treatments and four replications was used in the experiment. The 12 treatments were combinations of three cultivars and four levels of phosphorus fertilizer. The whole experimental field was an area of 48 m x 125 m. The details of the treatments are shown in Table 1 and the layout of the experiment is drawn in Figure 1.

4. Plant Cultivars Studied

Sugarcane cultivars planted in the experiment are:-

4.1 K 90-112

4.2 K 86-161

4.3 Phil 66-07

Table 1 Details of treatments with three sugarcane cultivars and four rates of phosphorus application

Treatment	Cultivars	Rate of Phosphorus	Symbols
1	K 90-112	no phosphorus fertilizer	V_1P_0
2	K 90-112	11.5 kilograms of P_2O_5/rai or 25 kg of TSP/rai	V_1P_1
3	K 90-112	16.1 kilograms of P_2O_5/rai or 35 kg of TSP/rai	V_1P_2
4	K 90-112	20.7 kilograms of P_2O_5/rai or 45 kg of TSP/rai	V_1P_3
5	K 86-161	no phosphorus fertilizer	V_2P_0
6	K 86-161	11.5 kilograms of P_2O_5/rai or 25 kg of TSP/rai	V_2P_1
7	K 86-161	16.1 kilograms of P_2O_5/rai or 35 kg of TSP/rai	V_2P_2
8	K 86-161	20.7 kilograms of P_2O_5/rai or 45 kg of TSP/rai	V_2P_3
9	Phil 66-07	no phosphorus fertilizer	V_3P_0
10	Phil 66-07	11.5 kilograms of P_2O_5/rai or 25 kg of TSP/rai	V_3P_1
11	Phil 66-07	16.1 kilograms of P_2O_5/rai or 35 kg of TSP/rai	V_3P_2
12	Phil 66-07	20.7 kilograms of P_2O_5/rai or 45 kg of TSP/rai	V_3P_3

where V_1 denotes K 90-112
V_2 denotes K 86-161
V_3 denotes Phil 66-07
P_0 denotes no phosphorus fertilizer
P_1 denotes 11.5 kilograms of P_2O_5/rai or 25 kg of TSP/rai
P_2 denotes 16.1 kilograms of P_2O_5/rai or 35 kg of TSP/rai
P_3 denotes 20.7 kilograms of P_2O_5/rai or 45 kg of TSP/rai

Note: 1 rai = 1,600 m^2
1 hectare = 6.25 rai

R1T4	R1T5	R1T10	R1T8	R1T11	R1T1
R1T6	R1T12	R1T2	R1T9	R1T3	R1T7

R2T6	R2T9	R2T3	R2T5	R2T2	R2T11
R2T4	R2T8	R2T12	R2T7	R2T10	R2T1

R3T9	R3T7	R3T1	R3T8	R3T12	R3T11
R3T3	R3T10	R3T5	R3T2	R3T6	R3T4

R4T5	R4T10	R4T6	R4T2	R4T11	R4T8
R4T1	R4T7	R4T3	R4T12	R4T4	R4T9

Figure 1 Layout of the phosphorus experimental trial

Where R denotes Replication
T denotes Treatment

5. Sources of Fertilizers

The sources of fertilizers in the study were:-

5.1 Nitrogen from ammonium sulphate (AMS) (21-0-0)

5.2 Phosphorus from triple super phosphate (TSP) (0-46-0)

5.3 Potassium from potassium chloride (KCl) (0-0-60)

6. Experimental Methods

Sugarcane is practically planted by using three-budded setts or whole stalks. However, in the experiment, single-budded setts were planted to control the plant population. Thus single-budded setts of three sugarcane cultivars were treated with fungicide, Byleton, and planted in polythene bags. The polythene bags used was made of polyethyl chloride with a dimension of 10 cm x 15 cm. Each bag was filled with 750 g of sandy soil taken from the experimental site. There was no application of fertilizer at this stage. Preparation of seedlings was performed on April 16, 1999.

Land preparation started at the beginning of April 1999 using three-disked plough to open up soil surface and dry the soil. Two weeks after first ploughing, ripping, ploughing and furrowing were performed to break hard pan and soil clumps,

and make rows for sugarcane planting. The experimental plot size was 10.4 m x 12 m or 124.8 m^2, consisting of 8 rows of cane at 12 m long, with 1.3 m interrow spacing.

On the 14th of June 1999, pesticide, Carbofuran, was applied to the rows before planting to control insects. Two-month old seedlings were transplanted to the field at the density of 24 seedlings per row with 0.5 m between plants. Furrow irrigation was done immediately after planting. Hand weeding was performed when necessary to control weeds.

The fertilizers were applied in split doses. Ammonium sulphate at 80 kg/rai (16.8 kg N/rai) was applied to all treatments three times, viz. 8 kg/rai (10%) at the time of planting (basal application), 24 kg/rai (30%) as side dressing at 70 days after transplanting (DAP) and at 48 kg/rai (60%) as top dressing at 130 DAP. Seventy percent of phosphorus fertilizer was applied in the form of triple superphosphate (TSP), at planting as basal application and the remaining 30% as side dressing at 70 DAP. Potassium chloride was applied at 20 kg/rai or 12 kg of K_2O/rai two times, viz. basal 14 kg/rai (70%) at planting and 6 kg/rai (30%) at 70 DAP. Dolomite, as a source of magnesium and calcium, was applied at 10 kg/rai as a basal fertilizer.

7. Data Recording

7.1 Rainfall Data

Rainfall data was collected from Sribunrueng Meteorology station, Changwat Nong Bua Lam Phu, located about 10 km from the trial (Appendix 1).

7.2 Soil Sampling Data

7.2.1 Soil sampling: Soil samples were collected before ploughing and after completion of the trial. Samples were collected from four levels of soil depth (0 - 15 cm, 15 - 30 cm, 30 - 60 cm and 60 - 90 cm) at five positions in each sub-plot.

7.2.2 Soil analysis: Air dried soil samples were used to analyse soil texture with hydrometer method, organic matter with wet oxidation method of Walkley and Black (1934), total nitrogen with Kjeldahl digestion method of Bremner (1960), available phosphorus was extracted with Bray II method of Bray and Kurtz (1945) and measured by spectrophotometer. Exchangeable potassium, calcium and

magnesium were extracted with 1N ammonium acetate solution and measured by atomic absorption spectrophotometer, soil pH with soil: water at 1:1 ratio using a pH meter, cation exchange capacity with 1N ammonium acetate (Peech, 1945). The results of soil are showed in Appendix 2.

7.3 Growth Parameters

Growth parameters were collected starting from the third month to harvesting. Sucrose accumulation was recorded from the sixth month to harvesting. Layout of sampling is showed in Figure 2.

7.3.1 Tillering: number of tillers arising from each stool was recorded during the 90^{th} day and converted to number per rai. Sugarcane stools were labeled from four middle rows, viz. 3^{rd}, 4^{th}, 5^{th} and 6^{th}, 2 stools per row or 8 stools per sub-plot. Tiller number was determined in each sub-plot from these labeled stools and calculated to tillers per rai.

7.3.2 Height of cane was measured on primary shoot, secondary shoot 1, and secondary shoot 2 for the first, second and third stalks, from the labeled stools. The length from the bottom to topmost visible dewlap was recorded.

7.3.3 Dry matter: samples were taken from the second and seventh rows, 3 stools per sampling. The stools were cut at the ground level, then separated into leaf sheaths, leaves, dry leaves and stalks. The plant parts were then weighed and samples were taken to be dried in an oven at 70° C for 48 hours to determine dry matter.

7.3.4 Crop Growth Rate: Crop Growth Rate (CGR) was measured using the formula:

$$CGR = \frac{W_2 - W_1}{t_2 - t_1} \times \frac{1}{P}$$

where CGR = Crop growth rate

W_1 = Total dry weight at harvest 1

W_2 = Total dry weight at harvest 2

t_1 = Time at harvest 1

t_2 = Time at harvest 2

P = Ground area covered by plant (m^2)

7.3.5 Leaf area (LA), leaf area index (LAI) and leaf area duration (LAD): Leaf area from the sampled stools was determined using a blue print paper method. Leaf area index and leaf area duration were calculated from following formulas:

$$LAI = \frac{LA}{P}$$

Where LAI = Leaf area index

LA = Leaf area (m^2)

P = Ground area covered by plant (m^2)

$$LAD = \frac{(L_2 + L_1) \times (t_2 - t_1)}{2}$$

Where L_1 = Leaf area (m^2) at harvest 1

L_2 = Leaf area (m^2) at harvest 2

t_1 = Time at harvest 1

t_2 = Time at harvest 2

7.3.6 Net assimilation rate: Net assimilation rate (NAR) was calculated using the following formula:

$$NAR = \frac{W_2 - W_1}{L_2 - L_1} \times \frac{\ln L_2 - \ln L_1}{t_2 - t_1}$$

where NAR = Net Assimilation Rate

W_1 = Total dry weight at harvest 1

W_2 = Total dry weight at harvest 2

t_1 = Time at harvest 1

t_2 = Time at harvest 2

L_1 = Leaf area (m^2) at harvest 1

L_2 = Leaf area (m^2) at harvest 2

$\ln L_1$ = Natural logarithm of leaf area at harvest

$\ln L_2$ = Natural logarithm of leaf area at harvest

7.4 Sucrose Accumulation

7.4.1 Quality and sucrose accumulation: at the sixth month until harvest, the second and third parts of cane sample, which were used for measuring dry matter, were taken from each sub-plot for analysis, one part for fibre analysis, the other for P_2O_5 in juice, brix, polarity reading, purity and CCS.

- Phosphate in juice was determined by colorimetric method with color developing by ammonium molybdate plus sulfuric acid and measured for the light absorption at 600 nm using a spectrophotometer (Spectonic21).
- Brix and polarity (Pol) readings were measured in cane juice by using an auto-refractometer and a polarimeter or polariscope respectively after the cane juice was added with lead acetate and the juice was filtered.
- Cane fibre content was obtained by washing shredded samples with hot water to remove sugar. Then, the samples were oven dried and weighed to determine %fibre content by the following formula:

$$\% \text{ Fibre (F)} = \frac{\text{Dry weight} \times 100}{\text{Fresh weight}}$$

- Commercial cane sugar (CCS) was calculated by the following formula:

$$CCS = \frac{3P}{2}\left[1 - \frac{F+5}{100}\right] - \frac{B}{2}\left[1 - \frac{F+3}{100}\right]$$

where P = % pol

B = % brix

F = % fibre

- Sugar yield was estimated from CCS and cane yield by the formula

$$\text{Sugar yield (kg/rai)} = \frac{\text{CCS} \times \text{cane yield (tonnes/rai)} \times 1{,}000}{100}$$

7.5 Nutrient Content in Tissue

Leaf analysis of phosphorus is a guide to nutrient status of sugarcane. Leaf samples were taken from 6 stools in the middle 4 rows (1 leaf per stool). The leaf samples were taken from the third top most visible dewlap leaf of the primary shoot of each stool. Then, the middle leaf blades were bulked after the mid-rib part was removed. They were then oven dried, grounded and were analysed for P content as per standard procedure. Leaves were sampled at 60, 80, 100 and 120 days after planting. From the fifth month, leaf samples were collected every month until harvesting (Anderson and Bowen, 1990).

The nutrient analysis from sugarcane parts was done by using plant tissue which was sampled for dry weight at harvesting. Stalks, leaves, leaf sheaths and dry leaves were separated, oven dried at $65\ ^{0}$c and analysed for nutrients.

The nitrogen content of plant samples was analysed by micro-Kjeldahl digestion and colorimetric determination of ammonium in the digests by an automated indophenol blue method (Schuman *et al.*, 1973). Phosphorus was extracted using wet oxidation (HNO_3 + Perchloric acid) and measured by a spectrophotometer. Potassium was also extracted using wet oxidation method and followed by atomic absorption spectrophotometer analysis.

7.6 Number of Stalks

Number of stalks per rai was counted from millable stalks at harvest and calculated from:

$$\text{Number of stalks per rai} = \frac{\text{Number of harvested stalks per plot x 1,600}}{52}$$

7.7 Stalk Length

At harvesting, stalk length was measured from bottom to natural breaking point from the 8 stools which were sampled for height and tiller number in each sub-plot.

7.8 Stalk Weight

Weight of stalks was calculated from:

$$\text{Weight of stalk} = \frac{\text{Total yield per plot (kg)}}{\text{Number of stalks per plot}}$$

7.9 Yield

Sugarcane yield per rai was calculated from the yield of the four middle rows of 10 m length with the area of 52 m^2. The measured cane stalk was obtained by cutting at the ground level and at natural breaking point. Then, the yield was calculated from:

$$\text{Yield per rai} = \frac{\text{Yield per sub-plot x 1,600}}{52}$$

7.10 Harvest Index (HI)

Harvest index of cane yield and harvest index of sugar yield were calculated using the following formulas:

$$\text{Harvest index of cane yield} = \frac{\text{Cane yield}}{\text{Total of fresh weight}}.$$

$$\text{Harvest index of sugar yield} = \frac{\text{Sugar yield}}{\text{Total dry weight}}.$$

7.11 Statistical Analysis

Statistical analysis was performed in a factorial randomized complete block design (Gomez and Gomez, 1984).

Stools	Rows 1	2	3	4	5	6	7	8
1	*	*	*	*	*	*	*	*
2	*	*	*	*	*	*	(*)7	*
3	*	(*)4	*	*	*	*	*	*
4	*(3)	*	(*)	*6	(*)	*5	(*)10	*
5	*	(*)8	*1	*7	*10	*4	*	*
6	*	*	*2	*8	*9	*3	(*)6	*
7	(*)3	(*)5	*3	*9	*8	*2	*	*
8	*	*	*4	*10	*7	*1	(*)9	*
9	*	(*)9	*5	(*)	*6	(*)	*	*
10	*	*	*	*1	*	*10	(*)6	*
11	*	(*)6	*6	*2	*5	*9	*	*
12	*	*	*7	*3	*4	*8	(*)10	*
13	*	(*)10	*8	*4	*3	*7	*	*
14	*	*	*9	*5	*2	*6	(*)7	*
15	(*)3	(*)7	*10	*	*1	*	*	*
16	*	*	(*)	*6	(*)	*5	(*)4	*
17	*	(*)4	*1	*7	*10	*4	*	*
18	*	*	*2	*8	*9	*3	(*)8	*
19	*	(*)8	*3	*9	*8	*2	*	(*)3
20	*	*	*4	*10	*7	*1	(*)5	*
21	*	(*)5	*5	(*)	*6	(*)	*	*
22	*	*	*	*	*	*	(*)9	*
23	*	*	*	*	*	*	*	*
24	*	*	*	*	*	*	*	*

Figure 2 Sampling layout of all analysed parameters

Where * denotes a stool of cane

n denotes n^{th} month after planting

(*)	denotes	a stool randomly selected for sampling stalk height and number of tillers from the third month after planting to harvesting
(*)n	denotes	a stool randomly selected for sampling dry matter, leaf area, sucrose accumulation and phosphate content in sugarcane juice at n^{th} month after planting
*n	denotes	a stool randomly selected for sampling the third leaf at n^{th} month after planting