CHAPTER 3
MATERIALS AND METHODS

1. Conceptual frame work

The factors affecting sugarcane productivity is depicted in Figure 1 which influences both soil and crop productivity. The soil productivity covers soil fertility (physical, chemical and biological properties), soil management (land preparation, fertilization, amendment, mulching and erosion control) and land resources (parent material and topography). The crop (sugarcane) productivity covers factors like variety (genetic and physiology) and cane management (seedcane, planting, weed, diseases, pest, harvesting and ratooning management). All components are affected by environmental factors (precipitation, solar radiation, wind speed and pollutant).

For an efficient cane production, we need to have suitable environment with optimum soil productivity and proper crop productivity factors. However, in this experiment site though we had favorable environmental and crop productivity factors like sufficient annual rainfall amount and distribution, sunshine, recommended variety and appropriate field management practices but found problems on soil productivity. The soil properties before planting in this experiment were compared with standard soil suitability classification of Prammanee (2001) is presented in Table 1.

2. Experimental Methods

2.1 Site selection

The selected site represents Korat soil Series in Northeast Thailand. The main character of Korat series found in the middle terrace with slope of 2-6 percent with good drainage, sandy loam or brown loamy sand texture in surface soil, sandy clay loam or clay loam in subsoil. The chemical properties consisted of pH ranging from 5.0-6.0 for topsoil and 4.5-5.5 for subsoil, low in soil fertility, mainly used for production of crops like cassava, sugarcane, beans and natural forest. This site has a main problem of poor ratooning ability which is caused by low soil fertility (pH $_{H_2O}$ 5.56, available phosphorus 20.45 ppm, exchangeable potassium 0.14 $cmol_c\ kg^{-1}$, 0.79

$cmol_c$ kg^{-1} of calcium, 0.26 $cmol_c$ kg^{-1} of magnesium, 0.43% of organic matter and cation exchange capacity 4.33 $cmol_c$ kg^{-1}). Study plot had problems on ratoon establishment and crop could not be maintained for more than three years.

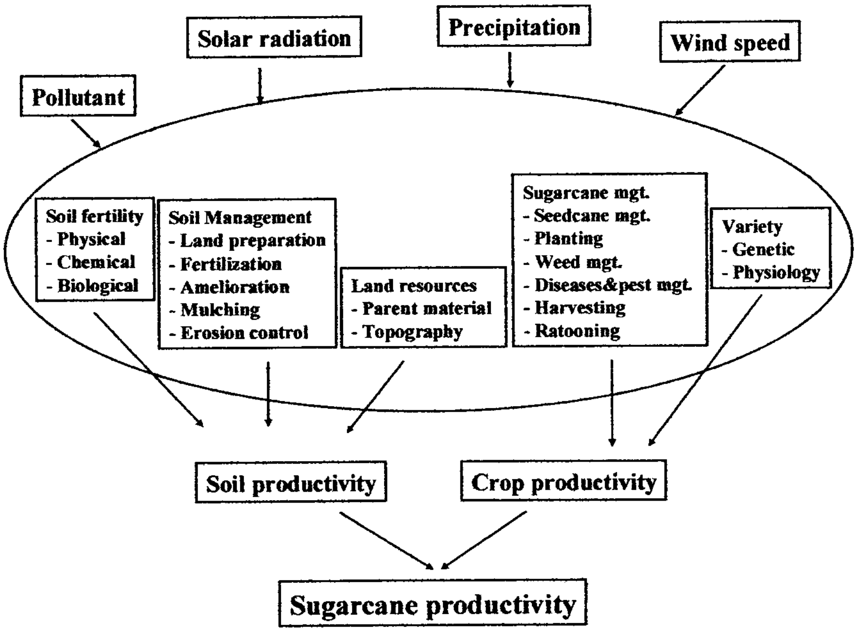

Figure 1 The factors affecting on sugarcane productivity

Table 1 The soil properties before sugarcane planting comparing with suitability classification

Properties	Requirement*	0-30 cm soil	30-60 cm soil	Classified
Bulk density (g/cm^3)	<1.5 in surface soil and <1.6 in subsurface soil	1.52	1.56	S
Textural class	Sandy loam to clay loam	Loamy sand	Loamy sand	U
pH (1:5 H_2O)	4.0-8.5	5.51	5.62	S
EC (dS/m)	<1.7	0.02	0.02	S
Avai. P (ppm)	10-20	25.11	15.79	S
Exch. K ($cmol_c$/kg)	0.24-0.71	0.13	0.14	U
Ca ($cmol_c$/kg)	0.55-1.25	0.76	0.82	S
Mg ($cmol_c$/kg)	0.10-0.25	0.26	0.25	S
OM (%)	2-4	0.47	0.38	U
CEC ($cmol_c$/kg)	> 15	4.19	4.47	U

Remarks: * Prammanee, 2001, S = Suitable and U = Unsuitable

2.2 Site description

The study site was located on latitude 12° 17′ 51′′ E, longitude 102° 17′ 51′′ N and altitude 160m above mean sea level. The annual rainfall was collected using 8 inches standard rain gauge of Mitr Kalasin Sugar mill station, Kuchinarai district, Kalasin province about 2 kilometers from the experimental field during the experimental period (24 February 2003 to 13 March 2004) was 1,624 millimeters. From the graph illustrated in Figure 2, we found three peaks of rainfall i.e. March, June and September or corresponding to 1, 4 and 7 months after planting cane. The maximum rainfall was recorded on 31 August 2003 with 99 millimeters and total 138 rain days/annum was recorded. Study plot was earlier planted with cane for two years with average yield of 62.5 t ha^{-1} in plant cane and 43.75 t ha^{-1} in first ratoon cane. The average rooting depth during the first ratoon was 80 cm. Soil properties before planting consisted of high bulk density, loamy sand texture, acidic soil, low organic matter, moderate available phosphorus, low potassium and low cation exchange capacity. This can be classified as low fertile soil and unsuitable for cane planting when compared with suitability for sugarcane planting index of Prammanee (2001). Soil properties in the experimental plots before application of the amendments were homogeneity with smooth slope. It is clear from Table 1 that soil is deficient in exchangeable potassium, organic matter and CEC with improper texture with loamy sand texture of Korat soil series (Kt: Oxic Paleustults, fine-loamy, siliceous) in field company area of Mitr Kalasin Sugar factory, Kuchinarai district, Kalasin province in Northeast Thailand.

The important problems of texture, exchangeable potassium, organic matter and cation exchange capacity (soil fertility factor) which can be solved by soil management techniques. The organic materials amendment with especially by-product of sugar manufacture and clay soil (2:1 clay) aimed at increasing exchangeable potassium, organic matter and CEC. The addition of organic materials can be effective but short-lived in tropical environments, requires large quantities and regular additions. An alternative approach is to increase nutrient retention properties where addition and incorporation of high-activity clay has been shown to permanently increase the CEC of the soil and provide positive yield benefits (Noble *et al.*, 2001; Noble *et al.*, 2003 and Noble *et al.*, 2004).

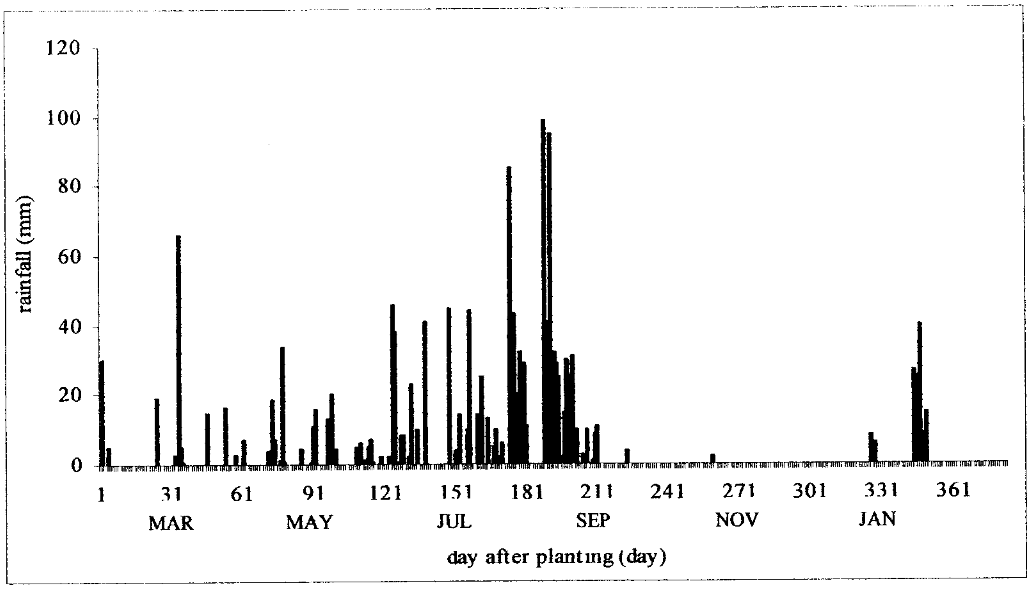

Figure 2 Daily rainfall pattern during February 2003 to February 2004 of Mitr Kalasin Sugar mill station

2.3 Site preparation

After the harvest of old ratoon cane in 2000, the land was left fallow for two years. The first land preparation was ploughed with three disc of Ford 6640 tractor to turn over soil surface and control weed by uprooting and allowing it to dry for a couple of weeks. After breaking the clods to smaller size suitable for air and water permeability and root activity using seven discs plough, the furrows were made using two discs plough with row spacing 1.2 meter and 40 cm deep.

2.4 Methods of amendment

The present work consisted of two field experiments on the methods i.e. conventional practice on soil amendment was broadcast and an alternative method was banding application. The banding method was applied materials at the base of sugarcane furrow for more efficiency of plant nutrients utilization, can apply for small farmer implement and apply for sugarcane planter. Both methods was used types of materials *viz.*, organic materials and clay soil as presented in Table 2 and described below.

Table 2 Treatments in two field experiments in different methods and types of materials amendment

Broadcast	Banding	Symbol
1. Control	1. Control	Control
2. Chemical fertilizer (CF) [1]	2. Chemical fertilizer (½ CF) [2]	CF
3. Clay soil 25 t ha^{-1} + CF	3. Clay soil 25 t ha^{-1} + ½ CF	CS_25
4. Clay soil 50 t ha^{-1} + CF	4. Clay soil 50 t ha^{-1} + ½ CF	CS_50
5. Clay soil 75 t ha^{-1} + CF	5. Clay soil 75 t ha^{-1} + ½ CF	CS_75
6. Filter cake 50 t ha^{-1} + CF	6. Filter cake 50 t ha^{-1} + ½ CF	FC
7. Cattle manure 25 t ha^{-1} + CF	7. Cattle manure 25 t ha^{-1} + ½ CF	CM
8. Bagasse 12.5 t ha^{-1} + CF	8. Bagasse 12.5 t ha^{-1} + ½ CF	BG

Remarks: [1] Chemical fertilizer rate 50-50-25 kgN-P_2O_5-K_2O ha^{-1}

[2] Chemical fertilizer rate 25-25-12.5 kgN-P_2O_5-K_2O ha^{-1}

2.4.1 Broadcast method

This trial was conducted to study the effect of organic materials (cattle manure, filter cake and bagasse) and clay soil on sugarcane yield and quality. Plot size was 400 m^2 (20x 20 meter) and conventional chemical fertilizer with addition of organic materials was used. The broadcasting was done by weighing materials in each plot belonging to the treatment and broadcasting before incorporation with seven discs plough, including clay soil (CS) @ 0, 25, 50 and 75 t ha^{-1}, filter cake (FC) @ 50 t ha^{-1}, cattle manure (CM) @ 25 t ha^{-1} and bagasse @ 12.5 t ha^{-1}, respectively. The conventional chemical fertilizer at the rate of 50-50-25 kg N-P_2O_5-K_2O ha^{-1} was applied in furrow as basal fertilizer. From the homogeneity of soil properties at before planting in uniform area, so the broadcasting trial was laid out in completely randomized design (CRD). The data collection was done in four replications (Figure 3) which can compare the effect of treatments on soil properties and plant growth within four replications.

2.4.2 Banding method

This trial was conducted to study the effect of organic materials (cattle manure, filter cake and bagasse) and clay soil on sugarcane yield and quality using banding of materials in the furrow. Plot size was 115.2 m^2 (8 rows, 1.2 meter spacing width and 12 meter length). The banding of materials at the base of furrow was done after furrow and half the rate of chemical fertilizer (25-25-12.5 kg N-P_2O_5-K_2O ha^{-1}) was applied in the furrow as the basal fertilizer. This experiment was laid on randomized complete block design (RCBD) with four replications.

2.5 Materials preparation

Organic materials used in this experiment are, cattle manure from cattle farm in Kuchinarai district, filter cake and bagasse from Mitr Kalasin Sugar mill, Kuchinarai district, Kalasin province and clay soil (2:1 smectite clay) of Kaset Thanarat company, Lop Buri province. The rate of application in each plot was calculated using row spacing, row length and number of rows for application area. Weighing materials and basal fertilizer for each plot and apply in corresponding plot.

2.6 Seedcane preparation

The diseases, pest, drought and infertile soil tolerance variety selected is Kanchanaburi (K) 88-92. Seed cane was prepared by cutting the cane into three budded setts. Seed sett counted into the bag for easy planting in each row (66 setts/row in broadcast method and 33 setts/row in banding method).

2.7 Crop maintenance

Both trials were irrigated before planting and top dressed using chemical fertilizer @ 68.75-15.625-56.25 kg N-P_2O_5-K_2O ha^{-1} at three months after planting. Manual weeding was taken up thrice (3, 5 and 7 months after planting).

2.8 Experimental period

The field study was conducted during February 2003 to March 2004 and the samples were collected and analyzed later.

3. Data collection

The data was recorded on ameliorant, soil, plant and rainfall as detailed below.

3.1 Amendment materials

3.1.1 Sampling

All materials samples of 2 kg were collected before amendment in the field.

3.1.2 Analysis

Air dried and sieved (2 mm) material samples were used to analyze chemical properties *viz.*,

- pH (1:5 H_2O) using pH meter (Black, 1965)
- Organic matter was determined using wet oxidation method of Walkley and Black (Black, 1965)
- Total nitrogen were analyzed using Kjeldhal method (Black, 1965)
- Total phosphorus measured by spectrophotometer (Bray and Kurtz, 1945)
- Total potassium were determined with flame photometer (Black, 1965)
- Calcium and magnesium were determined after digestion using atomic absorption spectrophotometer (Cresser and Parsons, 1979)
- Cation exchange capacity (CEC) using Metson method (Peech, 1945)

3.2 Soil

3.2.1 Soil sampling in plant cane plots

Soil samples were collected from 6 rows and 6 meter length with 4 replications in broadcast trial (Figure 3), and 6 middle rows and 12 meter length of each replication in banding trial (Figure 4). Samples were collected from two levels of soil depth (0-30 cm and 30-60 cm) at three positions in sugarcane row of sub-plot and then composite sample for each depth. Undisturbed soil samples with 2 replications in each subplot was collected for bulk density measurement. Air dried of disturbed soil and sieved (2 mm) was used to analyze chemical properties. Soil samples were collected four times i.e. before planting, 4^{th}, 8^{th} months and at harvest (12 months) after planting respectively. The soil sampling in 4 months corresponding to growth stage of sugarcane i.e. tillering, elongation stage and harvest and without effect of side dressing fertilizer. In addition, soil samples at 12 months after planting was collected after harvest about 1 week.

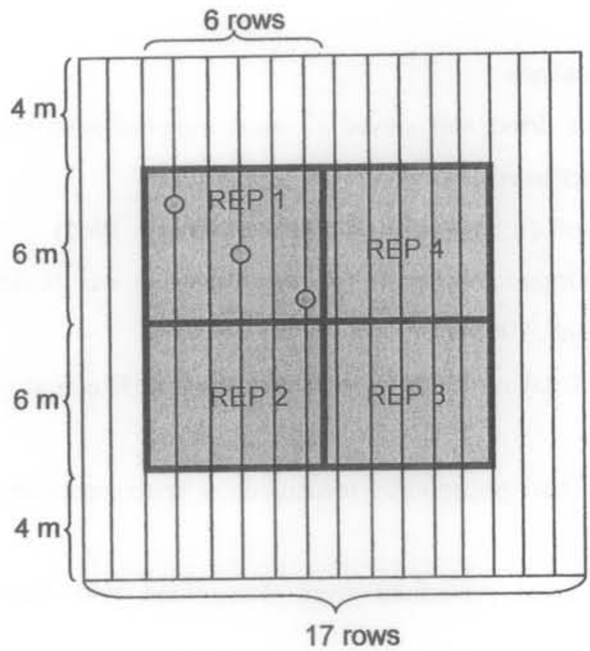

Figure 3 Diagram of soil sampling in broadcast trial in each treatment of plant cane

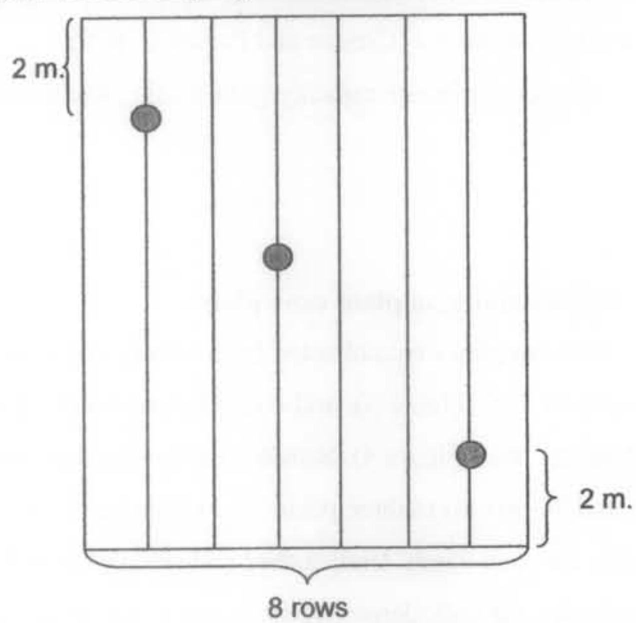

Figure 4 Diagram of soil sampling in banding trial in each replication of plant cane

3.2.2 Analysis in plant cane

Soil analyzes were carried out for physical and chemical properties as below.

- Bulk density was measured using core method (Brady, 1984)
- Particle size distribution was determined of pretreatment soil samples and measuring by pipette method (Dewis and Freitas, 1970)
- Soil moisture using gravimetric method (Gardner, 1956)
- Aggregate stability using wet sieving method (Black, 1965)
- Soil reaction was measured as pH (1:5 H_2O) using pH meter (Black, 1965)
- Electrical conductivity was measured 1:5 H_2O using conductivity meter (Jackson, 1958)
- Available phosphorus using BrayII method and measured by spectrophotometer (Bray and Kurtz, 1945)
- Exchangeable potassium, calcium and magnesium commonly used ammonium acetate pH 7 extraction and estimated using flame emission (Jackson, 1958; Chapman and Pratt, 1982) and atomic absorption spectrophotometry (AAS) (Jackson, 1958; Chapman and Pratt, 1982; Watson and Isaac, 1990 and Wright and Stuczynski, 1996)
- Organic matter was determined using wet oxidation method of Walkley and Black (Black, 1965)
- Cation exchange capacity (CEC) using Metson method (Peech, 1945)

3.2.3 Soil sampling in ratoon cane

Soil samples were collected in second ratoon cane (2.5 years after planting) that for confirm data in plant cane with more soil sampling points. Broadcast trial was collected within 4 replications of 6 rows and 6 meter length (Figure 5). Banding trial where samples were collected in 6 middle rows and 12 meter length (Figure 6). One pit in each row (diagonal path) with 6 pits in subplot and collected at two levels of soil depth (20 cm upper the transition zone and 20 cm lower the transition zone) as illustrated in Figure 7. The transition zone (diagnostic zone between topsoil and subsoil) was found at about 25 cm depth from soil surface which can observe with different soil colors. In addition, transition zone was separated of disturbed and undisturbed soil layers due to land preparation process. Composite samples in each depth by equal weight of soil sample and mixed properly, air dried and sieved through 2 mm.

3.2.4 Soil Analysis in ratoon cane

Air-dried soil samples were used for determining in physical and chemical properties *viz.*

- Particle size distribution was determined of pretreatment soil samples and measuring by pipette method (Dewis and Freitas, 1970)

- Soil reaction (pH 1:5 H_2O and 1:5 of 0.01 *M* $CaCl_2$) using pH meter (Black, 1965)

- Organic matter content was analyzed using wet digestion method of Walkley and Black (Black, 1965)

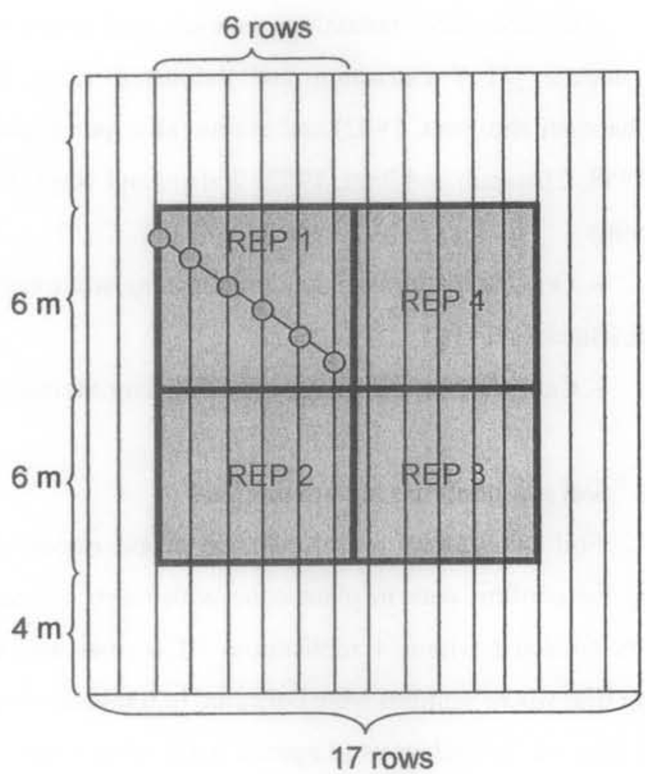

Figure 5 Soil sampling in broadcast trial in each treatment in ratoon cane

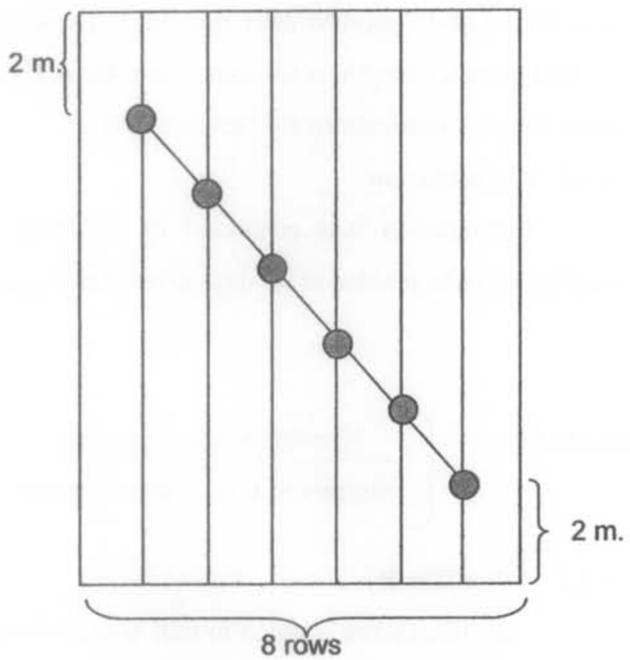

Figure 6 Soil sampling in subplot of banding trial in ratoon cane

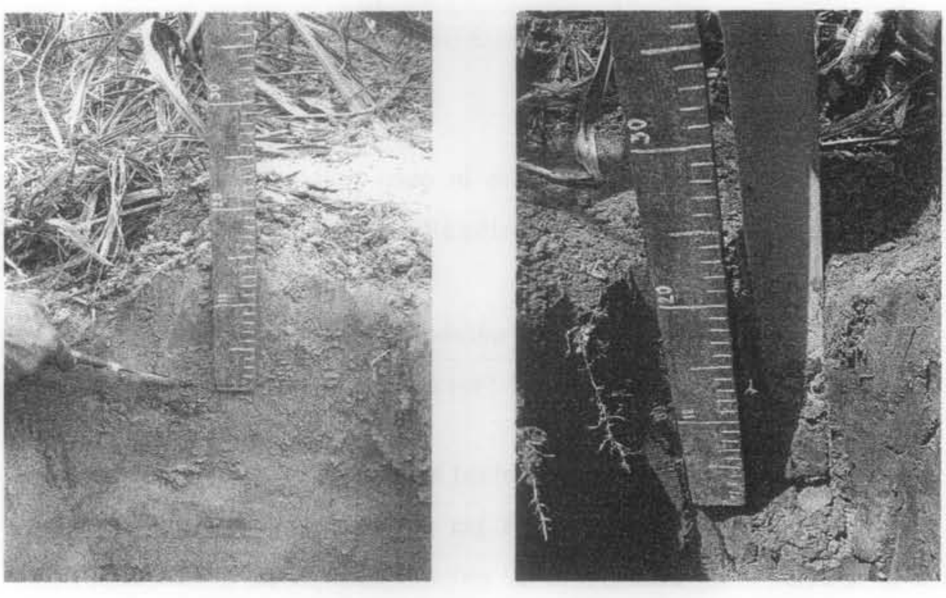

Figure 7 Soil sampling according to transition zone in ratoon cane

3.3 Plant

Sugarcane growth parameters were collected at three stages of growth. First is early growth (germinate to 3 months), mid growth (4-7 months after planting) and at harvest/ripening period (8-12 months after planting). These parameters were recorded from 6 rows and 6 meter length in all four replications for broadcast trial, and 6 rows and 12 meter length 4 replications for banding trial.

3.3.1 Germination

Germination was estimated by counting the total buds germinated against number of buds planted at 35 days after planting and expressed in percentage.

$$\% \text{ germination} = \left[\frac{\text{Number of shoot per row}}{\text{Number of buds planted per row}}\right] \times 100$$

3.3.2 Tiller density

All tillers were counted in unit area and expressed as tiller per hectare every month during 5 to 12 months after planting.

$$\text{Tillering (tiller ha}^{-1}) = \left[\frac{\text{number of tiller}}{\text{Plot size (m}^2)}\right] \times 10{,}000$$

3.3.3 Stool density

The number of stools in each subplot was counted and converted to stool per hectare during 3 to 12 months after planting.

$$\text{Stool density (stool ha}^{-1}) = \left[\frac{\text{number of stool}}{\text{Plot size (m}^2)}\right] \times 10{,}000$$

3.3.4 Stalk per stool and plant height

The number of stalk per stool and primary stalk height were collected in selected stool during 3 to 12 months after planting. The selected stools (once in middle of every row) were used to measure primary stalk height (from bottom to top most visible dewlap) and number of tiller per stool.

3.3.5 Stalk length, stalk diameter and stalk weight

The last stage is ripening stage or late growth stage during which we recorded stalk length, stalk diameter and stalk weight during 8 to 12 months after planting. Six stalk samples were taken from outer two rows. The length of stalks were measured from the bottom of stalk to natural breaking point, stalk diameter was measured at the mid of stalk and stalks weighed to record stalk weight.

3.3.6 Stool biomass at harvesting

The sugarcane stool biomass was recorded using eight parameters *viz.*, weight of dead leaf, weight of leaf sheath and top of green leaf, fresh weight of stalk, fresh weight of root, fresh weight of stubble, dry weight of root, dry weight of stubble and root length at harvest. These parameters were recorded from a single stool on area one square meter in 2 replications for each treatment.

3.3.7 Commercial cane sugar (C.C.S.)

Six stalk samples collected from outer two rows starting from 8 months onwards till harvest were used for estimating quality parameters. These samples were used for extracting juice. Brix and polarity (Pol) were recorded and the shredded fiber was washed to estimate fiber percent in each sample. Brix was measured using refractometer in laboratory to measure solid in solution (cane juice). The polarity was measured as transmittance using polarimeter or polariscope after adding lead acetate and filtering cane juice. The commercial cane sugar was calculated by using the formula:

$$\%\ \text{Fiber (F)} = \left[\frac{\text{Dry weight after washed}}{\text{Fresh weight before wash}}\right] \times 100$$

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

where; P = % Pol

B = % Brix

F = % Fibre

3.3.8 Millable cane

Millable stalks were counted in each plot and expressed as millable stalks per hectare using

$$\text{Millable cane (stalks ha}^{-1}) = \left[\frac{\text{number of stalk}}{\text{Plot size (m}^2)} \right] \times 10{,}000$$

3.3.9 Yield

This important trait was measured at harvest in each plot. The cane was cut at the bottom at ground level and top at top visible dewlap. The yield data was collected from 6 rows and 6 meter length (43.2 m^2) in broadcast plot and middle six rows and 12 meter length (86.4 m^2) in banding plot.

$$\text{Yield (t ha}^{-1}) = \left[\frac{\text{weight of cane per plot}}{\text{Plot size (m}^2)} \right] \times 10{,}000$$

3.3.10 Sugar yield

The sugar yield was estimated from C.C.S. and cane yield using formula:

$$\text{Sugar yield (t ha}^{-1}) = \frac{\text{C.C.S. x cane yield (t ha}^{-1})}{100}$$

3.3.11 Ratoon establishment

In ratoon cane after stubble shaving and chemical fertilizer application at the rate of 50-50-25 Kg N-P_2O_5-K_2O ha^{-1} and germination was checked after seven days. The ratoon establishment per hectare was calculated using formula:

$$\text{Ratoon establishment (shoot ha}^{-1}) = \left[\frac{\text{number of shoots}}{\text{Plot size (m}^2)} \right] \times 10{,}000$$

3.3.12 Nutrient concentration in third visible dewlap leaf

Leaf analysis was carried out to estimate nutrient content of the plant tissue. The relationship between plant nutrients and yield response can provide the relevant value for balanced nutrition. The crop to be analyzed was actively growing at the time of 8 months old. First and second stool of each row were selected for leaf sampling. The mid section from the third visible dewlap leaf, with the mid-rib removed was used for foliar analysis. A total of at least 20 leaves were collected per sample. Samples were kept in clean paper bag and dried at 70° C (Calcino *et al.*, 2000). Total nitrogen content analysis using Kjeldhal method (Black, 1965). Total phosphorus by digesting with nitric, perchloric and sulfuric acid (5:2:1) using yellow method and measured by spectrophotometer (Bray and Kurtz, 1945). Total potassium was estimated using digestion and flame photometer measurement (Cottenie, 1980). Calcium and magnesium were digested and measured using atomic absorption spectrophotometer (Cresser and Parsons, 1979).

4. Data and statistical analysis

One factor ANOVA under CRD of broadcasting trial was used to analyze the main effect of the organic and clay material treatments. One factor ANOVA under RCBD of banding trial was used to analyze the main effect of the organic and clay material treatments. The Duncan multiple range test (DMRT) was used for mean comparisons. The relationship and regression analysis between soil, growth parameters, plant nutrient concentration and growth parameters were determined by statistic package SPSS 11.0 for windows.