CHAPTER III
MATERIALS AND METHODS

3.1 Experimental site description

The experiments were conducted under typical field conditions with 2 different soil textures.

The first location was at Suranaree University of Technology, Nakhon Ratchasima, Thailand during the 2018/19 and 2019/20 planting seasons. The soil texture was sandy clay loam (SCL). The experimental field was 230 meters above sea level at 14°52’37” N latitude and 102°0’21” E longitude.

The second location was at KI sugarcane field, Buachet, Surin, Thailand with the same planting seasons as the first location. The soil texture was loamy sand (LS). The experimental field was 200 meters above sea level at 14°30’44.4” N latitude and 103°54’23.2” E longitude.

3.1.1 Soil properties analysis

In both experiments, the soil samples were prepared for physical and chemical analysis before starting a new crop (Table 3.1). The sample was mixed well and used the quartering and coning method (Campos-M and Campos-C, 2017). Soil texture was determined by using a specific gravity technic by the hydrometer method (Bouyoucos, 1962). Bulk density was determined by the could method (Blake and Hartge, 1986). The soil AWHC was calculated from the field capacity (FC) and permanent wilting point (PWP) of the soil, which was determined by the pressure plate technic. Soil EC was determined with a 1:5 soil to water ratio by a conductivity meter. The pH was determined with a 1:1 soil–to–water ratio by pH meter. The organic matter was determined by the Walkley–Black acid digestion method (Walkley, 1947). The available P was determined by the Bray II method (Bray and Kurtz, 1945). Ammonium acetate and atomic absorption spectrophotometer were used to determine exchangeable K, Ca, and Mg (David, 1960).

Table 3.1 Physical and chemical properties of soil.

Soil properties	SCL soil	LS soil
Physical properties		
Sand (%)	46.2	77.2
Silt (%)	22.5	23.1
Clay (%)	32.3	9.7
Bulk density (g/cm3)	1.25	1.35
AWHC (mm/cm)	1.65	1.12
Chemical properties (2018/19)		
pH	6.68	5.34
EC (dS/m)	0.031	0.233
OM (%)	1.43	0.63
Av. P (ppm)	12.7	5.8
Ex. K (ppm)	63.0	35.5
Ca (ppm)	2,030	442
Mg (ppm)	196	92
Chemical properties (2019/20)		
pH	6.77	5.27
Ec (dS/m)	0.072	0.049
OM (%)	1.08	0.62
Av. P (ppm)	2.93	4.95
Ex. K (ppm)	74.2	31.9
Ca (ppm)	2,150	459
Mg (ppm)	212	101

AWHC: Available water holding capacity; EC: Electric conductivity; OM: Organic matter; Av. P: Available P; Ex. K: Exchangeable K; SCL: Sandy clay loam; LS: Loamy sand

3.2 Experimental design

The experiments were conducted in 2 planting seasons with different experimental designs. Khonkaen 3 variety was used in both locations, since it is the most important cultivar that planted 60-70% in Thailand cultivation. It produces good yield with long ratooning period and better sustain for disease (Tippayawat et al., 2012). Sugarcane was planted by double row practice, with the within-row spacing of 0.3 m and row spacing of 1.6 m.

The first–year experiment was conducted with the plant cane crop (PC) and treatments were arranged by Randomized Complete Block Design with 3 replications. Treatments were crop managements :

T1: Rainfed conditions

T2: Drip irrigation with soil fertilizer application

T3: Drip fertigation

The second–year experiment was conducted with the first ratoon crop (FRC). The residual management of PC was splitted into 2 managements of FRC. The experimental design was Split Plot Design with 3 replications. Treatments were:

Main plot: the residue crop managements from PC

M1: Rainfed conditions

M2: Drip irrigation with soil fertilizer application

M3: Drip fertigation

Subplot: the crop managements in FRC

S1: Rainfed conditions

S2: Drip fertigation

3.3 Fertilizer application

In both soil textures and 2 continually year practices, the fertilizer was applied based on the nutrient balance model (NB). The major fertilizer sources were Urea (46–0–0), Monoammonium phosphate (12–61–0), Diammonium phosphate (18–46–0), and Potassium chloride (0–0–60). In soil application, the fertilizers were applied twice in the 1st and 3rd months. In fertigation, the fertilizers were fertigated equally every 3 weeks with 6 times from 1st to 5th months. The fertilizers were mixed and diluted with the water at the fertilizer: water ratio of 1:10 and injected into the drip irrigation system by a venturi pump.

The fertilizer rates of the nutrient balance model were calculated based on the nutrient balance equation (1) as the same study of Wonprasaid et al. (2021)

$$NS = \frac{NR-(SAN-SM)}{NUE} \qquad (1)$$

Where NS is the nutrient supply (kg/rai), NR is the nutrient required for the target yield of 30 tons/rai, SAN is the amount of soil available nutrient that obtain from soil analysis (kg/ rai) (Table 3.2), SM is the minimum amount of soil nutrient (OM=1%, P=10 ppm and K=60 ppm for SCL soil; OM=0.5%, P=5 ppm and K=30 ppm for LS soil) and NUE is nutrient use efficiency for soil uptake (N=80% P=60% and K=80%) for both soils.

Table 3.2 Rate of fertilizers recommended based on water balance model in each soil textures.

Soil texture	Planting season	N (kg/rai)	P_2O_5 (kg/rai)	K_2O (kg/rai)
SCL	2018/19	16.0	12.0	25.0
	2019/20	16.0	12.0	25.0
LS	2018/19	22.0	16.0	35.0
	2019/20	22.0	16.0	35.0

3.4 Irrigation practice

In both year practices, the treatment of the rainfed condition was no water supplied and the drip irrigation treatments used the drip tape with the 2 l/hr./dropper and the dropper spacing of 30 cm. Water was irrigated based on the water balance model equation (2) (Pereira et al., 2020)

$$I=ET+DR+RO+\Delta W-P \qquad (2)$$

where I is the irrigation, ET is the evapotranspiration, DR is drainage, RO is the surface runoff, ΔW is the available water holding capacity (AWHC) and P is the precipitation.

The water was re-irrigated when the water content was reduced from FC (100 % AWHC) to a critical point (40 % AWHC). At 1-2 months of sugarcane age, the root depths of 10 cm and 20 cm were used to calculate the water requirement of sugarcane, respectively. After 2 months until the harvest was calculated at 30 cm root depth

Daily water consumption was calculated by the same method as Xie (2018) based on equation (3).

$$ETc = ETo \text{ x } Kc \qquad (3)$$

where ETc is the crop evapotranspiration (mm/day), ETo is the amount of water consumed by standard or reference crop (mm), and an average ETo for 10 years (2010–2020) was used in this experiment. Kc is the crop coefficient.

The water content in soil was predicted by the same method as Xie which is shown in equation (4)

$$SMCp = SMCp_{n-1} - \frac{Etc - Re}{Dr} \times 100 \qquad (4)$$

Where SMCp is the predicted soil moisture content (%), n is the day of prediction, Etc is the amount of crop requirement (mm/day), Re is the amount of effective rainfall (mm), and Dr is the root depth (mm)

3.5 Sampling and data measurement

The recommended sampling area for double-row practice for data collection was 4.8 m x 4 m (19.2 m^2) (Hassan et al., 2017)

3.5.1 Plant cane (2018/19 growing season)

1) The plant height was measured from the soil base to the plant top visible dewlap (TVD) at 2, 4, 6, and 12 MAP.

2) The number of shoots was counted in the recommended area as mentioned earlier at 2, 4, 6, and in 12 MAP (number of millable cane, NMC)

3) Leaf area was measured at 4 MAP by Leaf Area Meter and leaf area index (LAI) was calculated by the Trimble method (2022) as in equation (6)

$$LAI=\frac{LA}{GA} \qquad (6)$$

Where LAI is the leaf area index, LA is the leaf area(m^2) and GA is the ground area(m^2)

4) The leaf SPAD chlorophyll meter reading (SCMR) (SPAD unit) was measured at the 4 MAP by using a handheld portable chlorophyll meter (Minolta SPAD-502 Meter, Japan). The second fully expanded leaf from the Top Visible Dewlap (TVD) of each plant was used for these measurements, as previously described (Jangpromma et al., 2010). In brief, the data points were recorded at 6 positions along the length of the leaf blade and then the data points were averaged into a single value. Sugarcane was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and that interference from the veins and midribs was avoided.

5) The Leaf nutrient including N, P, K, Ca, and Mg were analyzed in a young fully expanded leaf (4^{th} leaf next from TVD) and samples were randomly collected from 10 plants at 6 MAP.

6) The stalk diameter (middle of the trunk) was measured by using a Vernier caliper from 10 canes in the recommended area at the 12 MAP.

7) Yield (tons/rai) was calculated from the harvest area of 4.8 m × 4 m (19.6 m^2) at the 12 MAP.

8) Total soluble solid (TSS) (^{o}Brix) was analyzed using a reflectometer.

9) Irrigation water use efficiency and fertilizer nutrient use efficiency were calculated during the harvest period.

10) Underground stubble fresh and dry weight in sub-soil after harvest was recorded in the 1 m^2 per replication at 12 MAP and was collected only in SCL soil.

11) Total RLD and RLD patterns of the PC were measured at 12 MAP only in the SCL soil. The auger method was used to collect the root samples. The auger consists of a coring tube with a diameter of 0.8 cm and a length of 80 cm which is designed to reduce compaction in the inner tube by improving the cutting edge and reducing the tube's thickness. Root samples were collected at the row center (0 cm), 20, 40, 60, and 80 cm apart from the planting line. They were taken at a depth of 80 cm and separated into 3 layers consisting of upper soil layers (0–20 cm), middle layers (21–40cm), and deeper layers (41–80 cm). Root samples of each layer were washed manually with water to

remove soil from the roots. Then they were analyzed to determine root length by program WinRHIZO 2013 basic, Reg, Pro & Arabidopsis for root measurement, Regent instrument Canada Inc. Root length density (RLD) was calculated as the same method of Faye et al. (2019) as in equation (7).

$$\mathrm{RLD}=\frac{\mathrm{RL}}{\mathrm{SV}} \qquad (7)$$

Where RLD is the root length density (cm/cm^3), RL is the root length (cm) and SV is the soil volume (cm^3).

3.5.2 First ratoon cane (2019/20 growing season)

1) Germination (%) was recorded at 1 MAH of the ratoon crop, the equation was modified from Chumphu et al. (2019), due to the time limit of 2 crops continually as followed by the equation (8)

$$\text{Germination (\%)}=\frac{\text{Number of stool germminated of the ratoon cane}}{\text{Number of stool harvested of the plant cane}} \text{ x } 100 \qquad (8)$$

2) Growth parameters, yield, and yield components including plant height, number of stalks, yield, TSS, and stalk diameter were collected and measured as the same methods as the PC.

3) Ratooning ability (RA) was modified from the equation of Olaoye, (2008), due to this experiment was conducted with the time limit for two crops as the equation (9)

$$\text{RA (\%)}=\frac{\mathrm{RC_i}}{\mathrm{PC}} \text{ x } 100 \qquad (9)$$

Where RA was a ratio of properties among ratoon crop and plant crop in percentage, RC is the yield of properties in ratoon crop, and PC is the yield of properties in PC.

4) Total RLD and RLD patterns were collected at 6 MAH with the same method as in PC.

3.6 Statistical analysis

All data were analyzed using General Linear Model (Agresti, 2014). In all analyses, mean values were compared using Duncan Multiple Range Test (DMRT), and the significant differences were tested at a p–value <0.05. The combined analysis was performed with two sites only for the PC data.