CHAPTER 3

MATERIALS AND METHODS

Modeling of sugarcane flowering consists of two research activities. The first activity was the generation of data set on sugarcane flowering event knowledge base. The second activity was the development of a computer program to predict flowering event using climatic and management factors.

Generation of Sugarcane Flowering Data Set

The development of sugarcane knowledge-base was conducted in the Multiple Cropping Center Station, Chiang Mai University, Suthep Sub district, Muang District, Chiang Mai Province during May 1997 to December 1998. The station is located at 18° 45′ N latitude and 98° 55′ E longitude (UTM 47Q, X496400, Y2077800). Four sugarcane varieties: namely; CP78-1628, K84-200, K88-92, and U-Thong2 were used in this study.

The study consists of two experiments. The first experiment was the study of phenological development of the plant cane type of the four cane varieties. The second experiment was the study of the effect of varies photoperiod on phenological development of both plant and first ratooned cane of the four varieties. Both experiments were done under field condition with ample supply of water and nutrients.

Design and Treatments

The planting method of both experiments was similar. A single node sugarcane stalk was planted in a plastic bag 7 cm high with 6-centimeter diameter, filled with planting materials. Transplanting was done when seedlings reach their 5^{th} leaf stage. Distance of plants within the row was 75 cm and distance of each row was 100 cm.

First Experiment

Planting date of the first experiment was 5 May 1997. The experimental design was a Completely Randomized Design with 50 samples for emergence. Ten samples in each of seven replications in four blocks were observed their leaf development. Treatments were four sugarcane varieties: namely; CP78-1628, K84-200, K88-92, and U-Thong 2.

Second Experiment

Planting date of the second experiment was 9 March 1998. Cutting date to be first ratooned cane was 7 March 1998. The experimental design was a Completely Randomized Design with 50 samples for emergence. For after emergence development, eight samples in two cane types of four varieties under four photoperiods were observed their leaf development. Treatments were as follows:

Cane type:	Plant and first ratooned canes.
Varieties:	(a) CP78-1628; (b) K84-200; (c) K88-92; and (d) U-Thong 2.
Photoperiods:	(a) natural daylength; (b) 14 h d^{-1}; (c) 15 h d^{-1}; and (d) 16 h d^{-1}.

Photoperiod treatments were imposed by extending the natural daylength after sunset and before sunrise using halogen lamps, which give the same spectral with incandescent (Vince-Prue and Canham, 1983). A 100% saran shade cloth was installed between sections (Figure 3-1). Minimum light intensity was 100 Lux (0.325 Wm^{-2}), which was sufficient for photoperiod respond. Photoperiod treatments began on June 21 until December 20, 1998 for the 15 and 16 hours treatments and on July 8, 1998 for the 14 hours treatment. Water and nutrient management was kept at ample condition.

Data Collection

Weather Data

Daily weather data include solar radiation, maximum and minimum air temperature and rainfall were monitored and stored using a LICOR logger system. Daylength at the experimental site were calculated using both CERES and CBM models (Forsythe et al., 1995).

Plant Data

Three apical meristems of the main stalk were destructively sampled to the laboratory twice a week during September until December 1998. The samples were observed for floral bud by plant dissection technique (Kaveeta et al., 1995) and enlarged apex was identified (Figure 3-2), which also signified the floral initiation date of the cultivar.

Emergence date, leaf tip emergence date, leaf fully expanded date, number of tiller at each leaf position, and panicle emergence date were also collected.

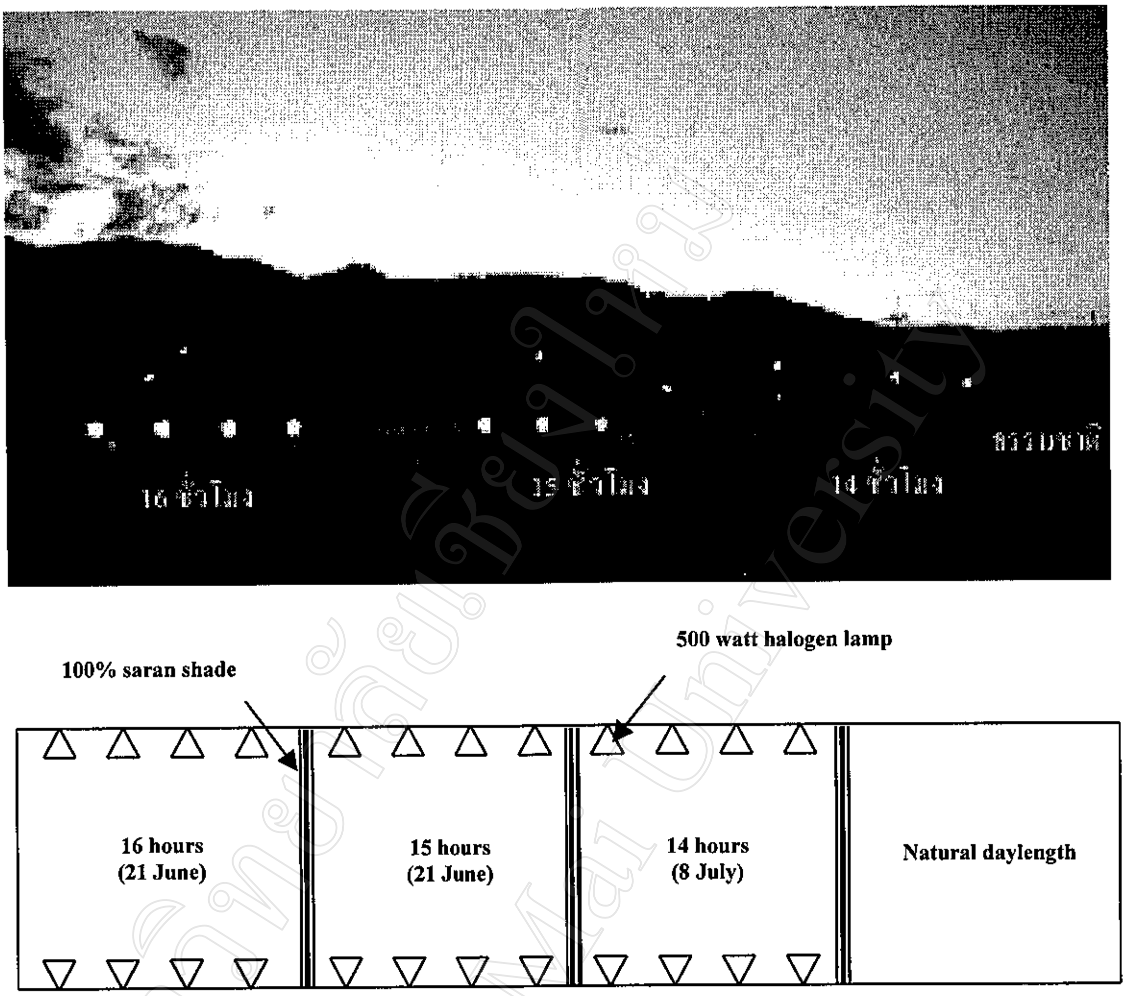

Figure 3-1 The experiment field layout for extended daylength experiment in sugarcane and photograph, Multiple Croping Center, Chiang Mai University.

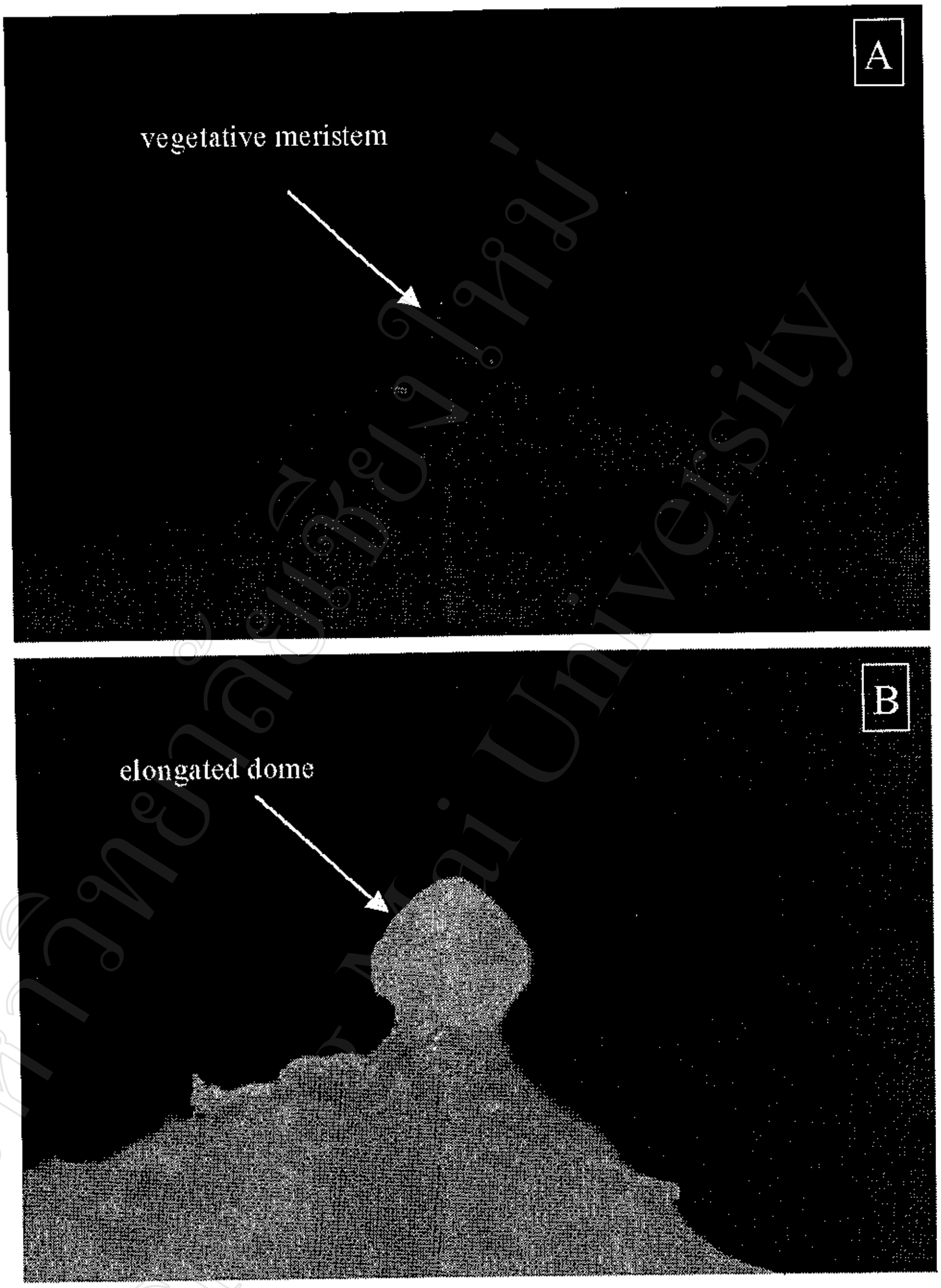

Figure 3-2 Photomicrographs (6×) of shoot dissection, showing the vegetative meristem (A); the change to an elongated dome, which indicates the shift to the flowering phase of a sugarcane plant (B).

Note: The stereomicroscope, under which the dissection was done, was provided by the central laboratory of Agricultural Faculty, Chiang Mai University, Chiang Mai, Thailand.

Data Analysis

Panicle Initiation Data Analysis

Determination of responsiveness to photoperiods of each sugarcane variety was done by fitting a line, using the least-square method, of relative response of development rate from emergence to panicle initiation as a function of photoperiods and tests for the significance of the slope were carried out. Varieties with a slope not significant at the 95% confidence level were considered insensitive to photoperiods, and their BVP values were the duration from emergence to panicle initiation, which was used in calculating the relative response of development rate of all treatments.

Sugarcane varieties showing a significant response were further analyzed to determine the BVP, the threshold between optimal and non-optimal photoperiod (P2O) and the relative induction rate slowed per hour photoperiod (PS) values with the following procedures.

The procedures consist of fitting of the horizon line, slope line and finding its intersection. Firstly, lower two photoperiods were used to fit a horizon line and other greater two photoperiods value to fit a slope line. The mean of relative response of development rate in natural photoperiod and 14 hour photoperiods for a variety was calculated (Y_1). A line was fitted by least square method for relative response of development rate as a function of photoperiods using the 15- and 16-hour values for the cultivar. A photoperiod value at the intersection of the line Y_1 and the fitted line were determined. If the photoperiod value at the intersection was greater than or equal to 14 hour, the intersection was considered to be the P2O for the cultivar.

PS was the slope of the fitted line and BVP was the duration used in calculating the relative response of development rate in natural and the 14-hour photoperiods.

In cases where the intersection was less than 14-hour photoperiod further analysis was necessary. A new mean of the relative response of development rate (Y_2) was calculated for the natural photoperiod only. The relative response of development rate value for other three photoperiods were pooled to calculate a photoperiod sensitivity line. The intersection of this line with the Y_2 line was determined, if the intersection was greater than the natural daylength at panicle initiation, this intersection was P2O. PS was the slope of the fitted line. BVP was the duration used in calculating the relative response of development rate under the natural photoperiod treatment.

Varieties with their intersections on daylength at panicle initiation or less were analyzed further. The relative response of development rate for four photoperiods were pooled and a least-squares line was fitted. A BVP value was assumed to be duration converted from the relative rate, at which predicted by this equation for the daylength at panicle initiation. A P2O value was taken as the daylength at panicle initiation, and the PS value was the slope of the line.

Others Plant Data Analysis

Other plant data, such as dates of emergence, leaf number on the main stem, tiller and panicle emergence data, were analyzed for their variance, the correlation and regression between variables by using Statistix 4.1program (Borland International, 1996).

Sugarcane Flowering Model

Model Assumptions

Due to various definition of photoperiod response, the following definitions were used in this research;

(i) "Threshold photoperiod" is the photoperiod, at which the threshold between optimal and non-optimal photoperiod. It is expressed as a cultivar specific coefficient (P2O).

(ii) "Critical photoperiod" is the photoperiod, where prevent flowering for absolute sensitivity type and take maximum floral induction duration for quantitative sensitivity type.

The critical assumptions related to the development respond to temperature and photoperiod are as follow;

(i) the rates of phasic, leaf, and initiated panicle development increased linearly with air temperature above a base temperature

(ii) the rate of panicle induction was assumed to be a function of both air temperature and photoperiod.

- The photoperiods, which are below the threshold photoperiod, have no delaying effect on panicle initiation. In optimal photoperiod condition, the panicle initiation rate depended only on air temperature, which was linearly related to the initiation rate.
- The photoperiods, which is above the threshold photoperiod, have delaying or preventing effect on panicle initiation based on the level of photoperiod and sensitivity of the variety. The effect of

photoperiods on the panicle initiation rates was expressed with a function of photoperiod and relative induction rate. The relative induction rate is calculated from division of minimum number of induction days, which occur under optimal photoperiod, and number of induction days at the photoperiod. The photoperiod was linearly negative relationship with relative induction rates. The relative induction rates decreased per hour photoperiod, called photoperiod sensitivity (PS), were assumed to be a constant for a variety.

(iii) Growing degree-day or daily thermal time system, as described in Chapter 2, is used to simulate phenology event. The base temperature of 16 °C was used for all phenological phases.

Model Inputs and Outputs

This model was designed to operate with weather data and crop genetic coefficients file format of DSSAT 3.5 system (Tsuji, 1994) and was written in Microsoft Visual Basic 5.0 (Appendix). The model used a daily incrementing time step and user are required to provide daily information of maximum and minimum air temperature from relevant weather file(s), which is DSSAT 3.5 system (*.wth). Latitude is also a required input from the weather file. Parameters to express the genotype characteristics are also necessary (Table 3-1).

Table 3-1 Sugarcane genetic coefficients used in the ScFM 1.0 model

Parameter	Code	Description
First Phyllochron Interval	PI1	GDD required for a leaf production of the first 14 leaves.
Second Phyllochron Interval	PI2	GDD required for a leaf production of after the 14th leaf.
Threshold Photoperiod	P2O	The photoperiod, below which is optimal for panicle induction.
Photoperiod Sensitivity	PS	Change of relative response of development rate per hour of photoperiod.
Minimum induction GDD	P22	GDD required for panicle initiation under the optimal photoperiod.
Panicle Emergence	PE	GDD required for panicle development since initiation to emergence.

These parameters are kept in crop genetic coefficient file of DSSAT 3.5 system (sccan980.cul file). Management information as planting date, harvesting date and sowing depth are also required inputs and user can interactively enter whole runing the model.

The simulation results are stored as DSSAT 3.5 simulation result format. The result file consists of information of the used weather station, the genotype specific parameter of the selected cultivar and the simulated data, which are daylength, cumulative GDD, sugarcane growing stage and total leaf number (Figure 3-3). The result will be shown up as text after simulation finished and could be graphically displayed and printed.

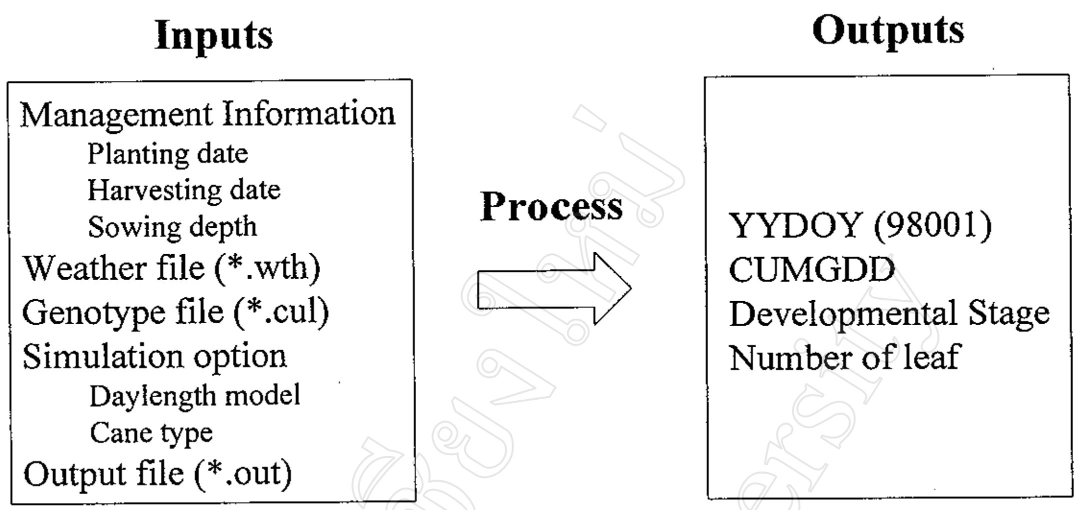

Figure 3-3 Model inputs requirement and its simulation results

Model Operations

This model operates on a daily incremental time step, which is executed from the planting to the harvesting dates (Figure 3-4). First, the growing degree-day or daily thermal time is calculated, from daily maximum and minimum temperature, which are read from the weather file. Then, daily daylength is calculated using a selected daylength simulation model. This model provides two daylength models; CBM and CERES model. Each model requires latitude in degrees and day of the year (jdate) (Forsythe et al., 1995), which is already converted by the program as input. The output of each model is hours of light for flat surfaces. The length of each day varies with location upon the earth and day of the year. Daylength also depends upon the definition one uses for the beginning and end of the day, and whether twilight is included in daylength. Table 3-2 shows the daylength definitions allow in the CBM model. The definition of daylength in the CERES model is fixed to be daylength as including the periods of civil twilight. Next, variables relevant to the present phase are calculated. The decision is made as to whether the next phenological phase has been reached. Finally, the simulated results are written in an output file, which its name is created or selected by the user.

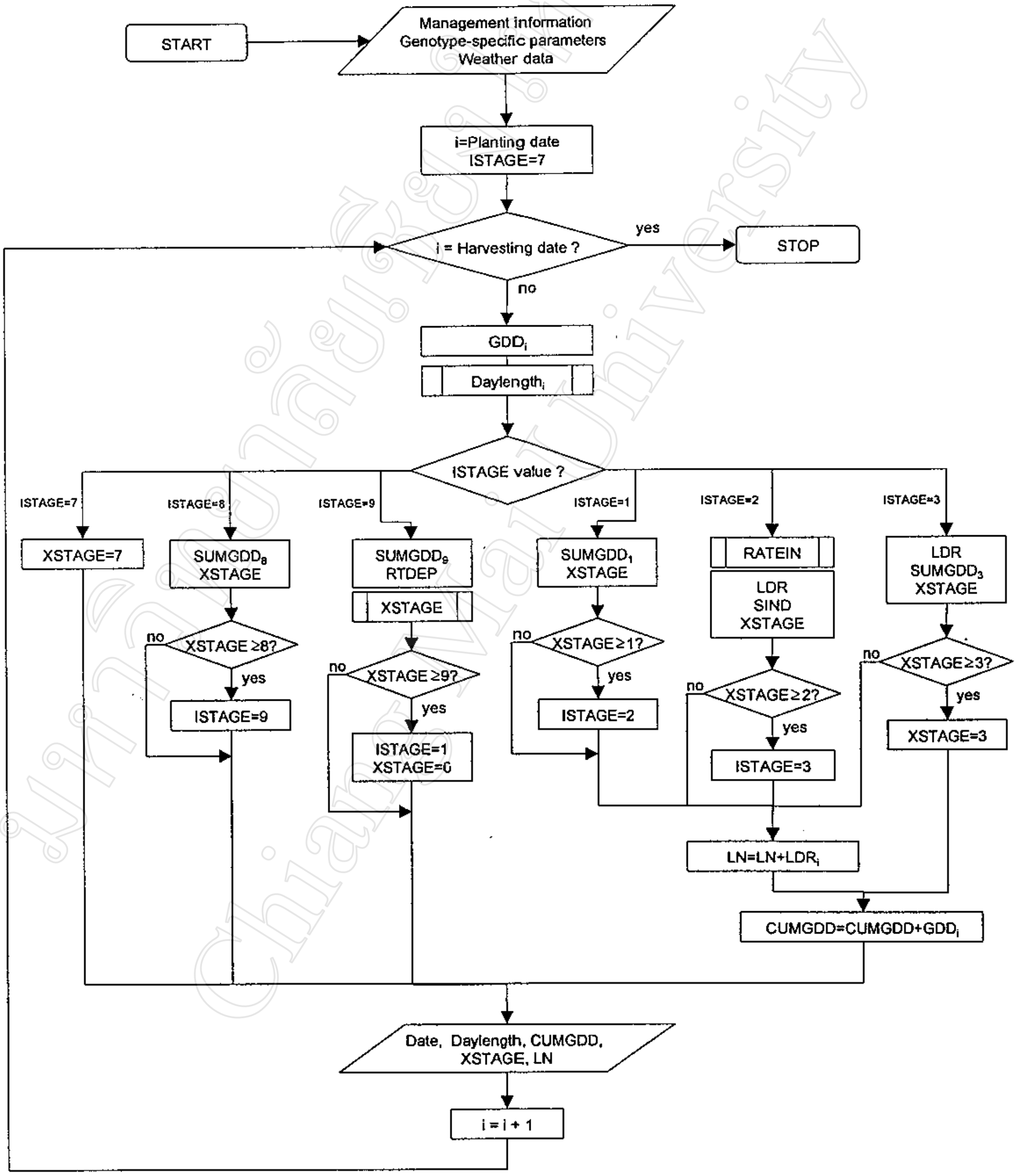

Figure 3-4 Flow chart of the sugarcane flowering model ScFM 1.0

Table 3-2 Daylength definitions defined by the position of the sun with respect to the horizon

Definitions	Daylength definition (with and without twilight)	*p*(degrees)
1	Sunrise/Sunset is when the center of the sun is even with the horizon	0.0
2	Sunrise/Sunset is when the top of the sun is even with horizon	0.26667
3	Sunrise/Sunset is when the top of the sun is apparently even with the horizon (US government definition)	0.8333
4	With civil twilight	6.0
5	With nautical twilight	12.0
6	With astronomical twilight	18.0

Source: Forsythe et al. (1995)

Sugarcane Development Stages

In the ScFM 1.0 model, the developmental phases of sugarcane plant were separately numbered, between aboveground and underground, with an identifying integer ISTAGE (Table 3-3). ISTAGE numbers 1 to 3 are the aboveground developmental phase; ISTAGE 7 to 9 are events in crop management and underground developmental phase. The developmental stage of sugarcane on a given day is represented in the model by a temporary variable XSTAGE.

Table 3-3 Sugarcane development phases

ISTAGE	Phase description
7	Planting
8	Planting to 50% Germination
9	50% Root germination to 50% Emergence
1	50% Emergence to 50% End of Juvenile or 14^{th} leaf fully expanded
2	50% End of Juvenile or 14^{th} leaf fully expanded to 50 % Panicle Initiation
3	50 % Panicle Initiation to 50 % Panicle Emergence

ISTAGE 7 Planting or Start of Simulation

The model is first initialized the XSTAGE variable as 7 on the first day of simulation or on the defined planting date.

ISTAGE 8 Planting to Root germination

The model assumes one day for root germination process of all sugarcane varieties. This assumes adequate soil moisture for root germination at the time of planting. After one day, value of the XSTAGE variable is then updated to 8. Then, ISTAGE is set to 9.

ISTAGE 9 Root germination to Emergence

For plant cane, the equation for prediction of this interval are taken from ThaiCane 1.0 model (Jintrawet et al., 1997b). Prior to emergence, root length (RTDEP, cm) increasing daily as a function of GDD, emergence occurs when summation of growing degree-day since Root germination ($SUMGDD_9$) reach P9, determined from sowing depth (SDEPTH, cm)

$$RTDEP = RTDEP + (0.15 \times GDD_i) \qquad (3.1)$$

$$P9 = 200 + (10 \times SDEPTH) \qquad (3.2)$$

$$XSTAGE = \frac{SUMGDD_9}{P9} \qquad (3.3)$$

For ratooned cane, the prediction equation 3.4 is modified from the equation 3.3, which is for plant cane. Emergence of ratooned cane occurs when summation of growing degree-day since Root germination ($SUMGDD_9$) reach 200, whose value is from the assumption that thermal requirement from harvest to appearance of the first leaf in ratoon crops was assumed to be 200 GDD (Robertson et. at., 1998).

$$XSTAGE = \frac{SUMGDD_9}{200} \qquad (3.4)$$

When the emergence occurs, the XSTAGE reaches 9, then, ISTAGE is set to 1.

ISTAGE 1 Emergence to End of Juvenile

After emergence, the plants begin to produce its leaves, so calculation of leaf development rate (LDR) (Equation 3.5), starts at this phase of any sugarcane varieties, as well as the CUMGDD variable. The model divides leaf development into two intervals. The first phyllochron interval (PI1) is used for the first 14 leaves, which its value is a genotype specific constant that kept in genotype file.

$$LDR = \frac{GDD_i}{PI1} \qquad (3.5)$$

For XSTAGE, it is set to be zero and then calculated from equation 3.6 which is from the assumption that the juvenile phase is not sensitive to photoperiod. The end of juvenile occurs when it showed four fully-exposed internode (Clements,

1975) that was observed at the 14th leaf fully expanded (Jintrawet et al., 1997a), which is controlled by temperature and varies with cultivars.

$$XSTAGE = \frac{SUMGDD_1}{14 \times PI1} \qquad (3.6)$$

At the end of the juvenile stage, XSTAGE reaches 1 and ISTAGE is updated to 2.

ISTAGE 2 End of Juvenile to Panicle Initiation

After end of juvenile, a sugarcane plant produce its leaves with other leaf development rates, which are calculated based on the second phyllochron interval (PI2), which used from the 15th leaf stage onward, and ready to be induced to initiate a panicle.

$$LDR = \frac{GDD_i}{PI2} \qquad (3.7)$$

The rate of panicle induction (RATEIN) is calculated as a function of daily daylength and daily temperature (equation 3.8)

$$RATEIN = F(DL) \times F(T) \qquad (3.8)$$

Where RATEIN is panicle induction rate on the day, F(DL) is the function of daylenght (equation), F(T) is the temperature function (equation 3.9).

$$F(DL) = 1 + PS \times (P2O - DL) \qquad (3.9)$$

Where DL is daylength, in hours; PS is the photoperiod sensitivity which is change of the relative rate of panicle induction per hour; P2O is the threshold photoperiod, below which is optimal for induction (i.e., the relative rate of induction is one if daylength equal to or less than P2O). The function of daylength is set to be zero if it is negative value. This situation occurs when daylengths are above the critical photoperiod. So, the photoperiod sensitivity determines the critical photoperiod, above which induction never occurs.

$$F(T) = \frac{GDD_i}{P22} \quad \text{.......(3.10)}$$

Where T is air temperature, GDD is daily growing degree day; P22 is the minimum GDD required for panicle induction, which is assumed to equal to the second phyllochron interval (PI2).

At the optimal daylength, where F(DL) is equal to one, inductive development progresses at it fastest rate and the duration is equal to the GDD required for a leaf (Clements, 1975).

$$SIND = SIND + RATEIN \quad \text{.......(3.11)}$$

$$XSTAGE = 1 + SIND \quad \text{.......(3.12)}$$

The F(DL) and F(T) are computed daily. Progress toward panicle initiation stage is computed by integrating RATEIN start from ISTAGE 2 with a temporary variable SIND, PI occurs when SIND reaches 1.0. At that time, XSTAGE reaches 2 and ISTAGE is updated to 3.

ISTAGE 3 Panicle Initiation to Panicle Emergence

Panicle emergence occurs when the summation of growing degree day since panicle initiation stage ($SUMGDD_3$) equals or exceeds PE. PE is a genotype specific constant, as defined in Table 3-1.

$$XSTAGE = 2 + \frac{SUMGDD_3}{PE} \quad \text{.......(3.13)}$$

During panicle initiation to panicle emergence, the initiation of new leaf ceases at panicle initiation stage, thereafter the initiated leaf has developed until the last leaf fully open as panicle emergence.

At panicle emergence, the developmental stage variable XSTAGE is updated to 3 and leaf expansion stops. The model stops simulating new leaf when XSTAGE variable equal or greater than 3, but daily daylength, growing degree day, and CUMGDD are daily calculated until the harvested date is reached.