# Chapter 6

## Changes in Physiology and Biochemistry of Litchi Plant when Flowering Under Low Temperature

### 6.1 Introduction

It was cleared in the result from chapter 3 and chapter 5 that low temperature (15/10°C) treatment for 38 day could promote flowering in litchi. Effects of low temperature on diminish the photosynthetic rate, transpiration rate and stomatal opening were also confirmed. At the same time low temperature effect on inflorescence panicles and plant hormones level were proved, especially the flowering of Z/ZR concentrations in terminal bud, bud, bark, wood, leaf, xylem exudates and leaf diffusate. Effect on IAA level and GAs requires, however confirmation of the relationship between the hormonal change and terminal bud development studied in chapter 5 could also not yet concluded clearly. Therefore, the further experiment must be conducted to enlighten the effect of low temperature on flowering of litchi tree, especially at the physiological and hormonal level. Morphological study of bud during cold treatment must be parallel followed to confirm the relationship between hormonal level and bud transformation.

Due to a limitation in research equipment, the experiments in chapter 3 and chapter 5 were conducted even though with the concentrations of different temperatures require (comparing 15/10°C day/night require with greenhouse temperature) but the interfering effect of difference in high intensity could not be totally excluded. A clear effect of only low temperature must be therefore repeated in the experiment in chapter 6 again.

Moreover, the result in bud-section in chapter 5 showed no morphological development even in 28 days cold treated plants, although a decrease in Z/ZR level was observed. It was resumed that effect of plant hormones on bud development may be not the immediate effect but rather time consuming process. The low level of Z/ZR at 28 days of cold treatment would show its effect on bud development in the later weeks. This must be confirmed.

In this chapter, therefore, the unsatisfied treatment conditions especially different temperature with the same light intensity will be improved. The development of terminal bud

in the late period of cold treatment up to during temperature rising period will be followed together with the hormonal changes. A better understanding on mechanism of physiological response of litchi when flowering under low temperature condition should be achieved.

**6.2 Material and methods**

One hundred and forty 'Hong Huay' litchi trees grown in 5 inches diameter plastic bag were sampled for the experiment. Standardization of plant material was relied on firstly leaf ages of 80 day-old mature leaves and secondly the third leaf form shoot apex, which with leaf chlorophyll values at ranges of 42-47 measured with the chlorophyll meter SPAD-502. The trees were divided into two groups for warm and cold treatment. For the warm temperature treatment, the trees were kept in a growth chamber and subjected to a diurnal day/night temperature of 35/27°C, which relevant to the average ambient temperatures during June - August 2002 (shown in chapter 3). In the case of the low temperature treatment, the trees in the growth chamber were exposed to diurnal day/night temperature of 15/10°C for 38 days. After 38 days of cool period, the temperature was then stepwise increased to 29.5/24°C within 14 days after the treatments. Data collection on changes in TNC, RS and hormonal concentrations as well as bud-section were done at day 35 of cold treatment and another 4 times (7 days interval) during temperature rising period, the experiments were conducted during April-August 2003 at Lampang Agricultural Research and Training Centre.

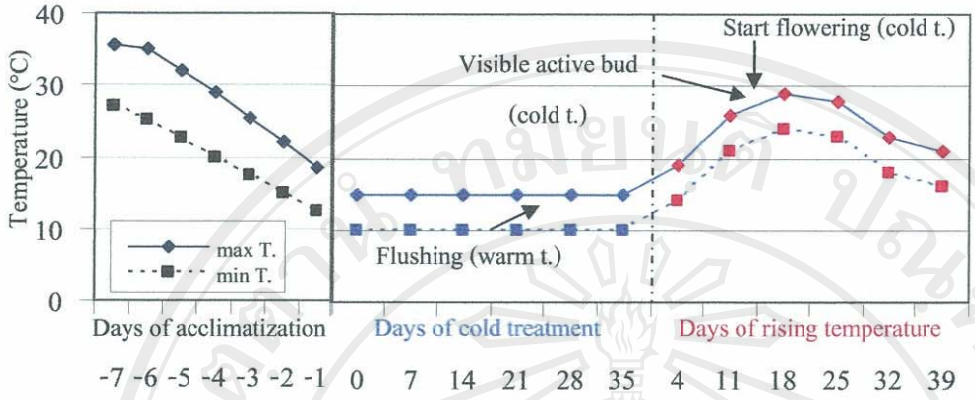



**Figure 6.1** Maximum and minimum of ambient temperature in the cold growth chamber during the acclimatization period, cold treatment and temperature increasing period

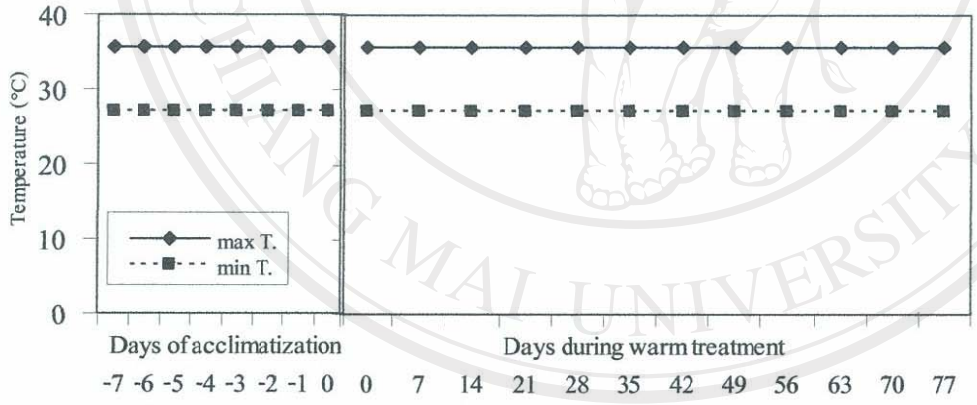



**Figure 6.2** Maximum and minimum temperatures in the warm growth chamber during the experiment period



### 6.2.1 Changes in terminal bud development

Five shoot tips per treatment were four times sampled at weekly interval during day 35 of cold treatment and day 4, 11 and 18 of temperature rising period. At the day 18 of temperature rising period the active buds started bursting and visualize, this called as "visible active bud" in this experiment. The sampled bud in the total amount of 40 days collected from warm and cold

treatment were studied for morphological change using paraffin embedded method as already described in chapter 5.

**6.2.2 Percentage and days to flowering after cold treatment**

Ten litchi trees (replications) were sampled for flowering observation. The collected data were the percentage and days to visible active buds, flowers and flushes as same as in chapter 5.

**6.2.3 Changes in leaf total chlorophyll**

Eighteen fully intact leaflets, the same leaves as for photosynthetic measurement, were investigated by using chlorophyll meter (Model SPAD-502, Minolta, Japan) during 11:00 to 11:20 o'clock. It was also calibrated with standard curve of chemical analysis values of total chlorophyll concentrations as described in chapter 5.

**6.2.4 Changes in leaf physiological activities**

Determination of physiological changes was performed on photosynthetic rate, transpiration rate, stomatal conductance, leaf temperature and photosynthetically active radiation. It was investigated both at weekly from the beginning of experiments until weeks 8 by measuring during 10:30 to 12:00 o'clock, and the diurnal changes at day 10, 24 of cold duration and day 3 of temperature rising period. All the measurements were done at 2 hours interval from 09:00 to 17:00 o'clock using the same procedure explained in chapter 5.

**6.2.5 Changes in carbohydrates content in leaves and roots**

Leaf and root samples were also collected for carbohydrate analysis at the same days as hormonal analysis studies and using the same procedures as described in chapter 3.

**6.2.6 Changes in endogenous hormonal contents in plant organs**

Fifteen trees per treatment were collected each time for hormonal analysis at day 35 of cold treatment and at day 4, 11 and 18 of temperature rising period. Specific plant parts, e.g. terminal bud plus two lateral buds, leaf, bark, wood, xylem exudates, and leaf

diffusate were analyzed for Z/ZR, i-Ado/i-Ade, IAA and $GA_{1, 3, 20}$ by using the RIA method at the University of Hohenheim, Germany as described in chapter 5.

## 6.3 Results

### 6.3.1 Percentage and days of flowering after cold treatment

The terminal buds of litchi trees exposure to warm temperature had 100% leaf flushing within 17-28 day of the experiment (Table 6.1). In contrast to low temperature treatment, when temperatures increased after 38 days of cool period, the first visible active buds were already observed at day 13 of temperature rising period, after that the complete (100%) bud transformation into visible inflorescence bud burst was found at day 17 (Figure 6.3). This stage was referred as "days to start flowering".

**Table 6.1** Effects of temperatures on leaf flushing and flowering

(10 replications)

| Parameters | Low temperature treatment | Warm temperature treatment |
|---|---|---|
| Days to flushing (days) | 0 b | 17 a |
| Percentage of flushing shoots (%) | 0 b | 100 a |
| Days to visible active bud (days) | 13 a | 0 b |
| Percentage of visible active buds (%) | 100 a | 0 b |
| Days to start flowering (days) | 17 a | 0 b |

a, b means in the same row followed with the same letter does not significant difference at $\alpha = 0.05$ by Lsd.

### 6.3.2 Development of terminal buds during cold treatment

Changes of terminal buds development at different day of cold treatment when studied under microcopy were shown in Figure 6.3. Under warm temperature, all the buds flushed into young leaves at day 17 of the treatment (Table 6.1). The terminal buds collected for morphological change studies were therefore arriving their ages at 17, 24, 31 and 38 days after flushing. The microscopy results showed the similar development of terminal buds at all ages as

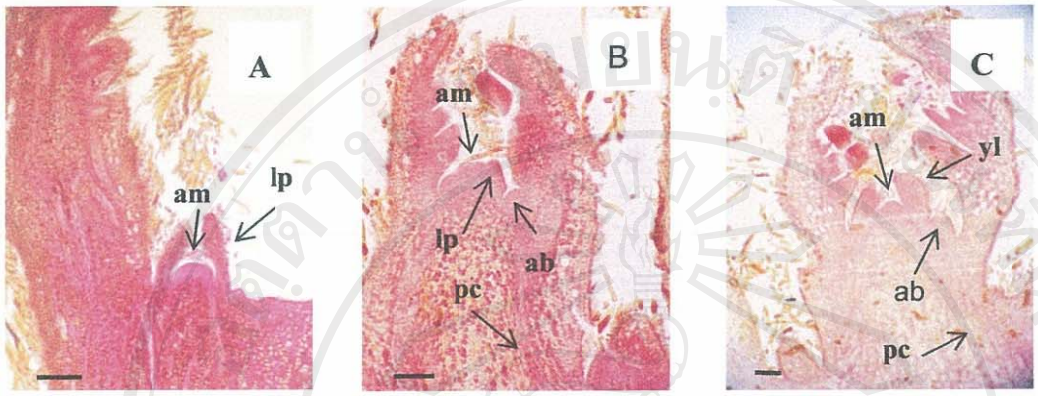
showing in Figure 6.3 A, 38 day–old bud after flushing. The vegetative terminal bud remained round shape at a dome part containing only leaf primordial and apical meristem.

Under low temperature treatment, at 35 days of cold treatment (Figure 6.3 B) the terminal bud remained dormant under normal observation but morphological change at tissue level could be detected under microscopy. Apical meristem started flattening with enlarging of leaf primordial and axillary bud. Procambium tissue, fundamental tissue for vascular bundle, which had darker color of staining, means the symbol of more active. By rising up the ambient temperature in the growth chamber for 4 days (Figure 6.3 C), procambium development was clearly detected, where as leaf primordial development to be young leaf together with the enlargement of axillary bud. At day 11 of temperature rising up a clear reproductive buds development were observed at the terminal and axillary buds, which supposed to be the differentiation of inflorescence meristem/bud development. At day 18 of temperature rising period (Figure 6.3 E) a clear bud-swelling could be visible and the same buds started flowering (bud bursting stage). Development of young inflorescence bud was completed containing of all necessary fundamental parts; apical meristem, leaf primordial, young leaf, florescence bud and procambium. The buds were then ready to further develop into the florescence protrusion. The terminal buds were collected at the same experimental day. The results were shown in Figure 6.3 A, which it had however young age due to the flushing under warm temperature.

38 days after flushing under warm temperature (35/27°C)

35 days of cold treatment (15/10°C)

4 days after rising t. of cold treatment (19.5/14.5°C)




11 days after rising t. of cold treatment (26/21°C)

18 days after rising t. of cold treatment (29.5/24°C)

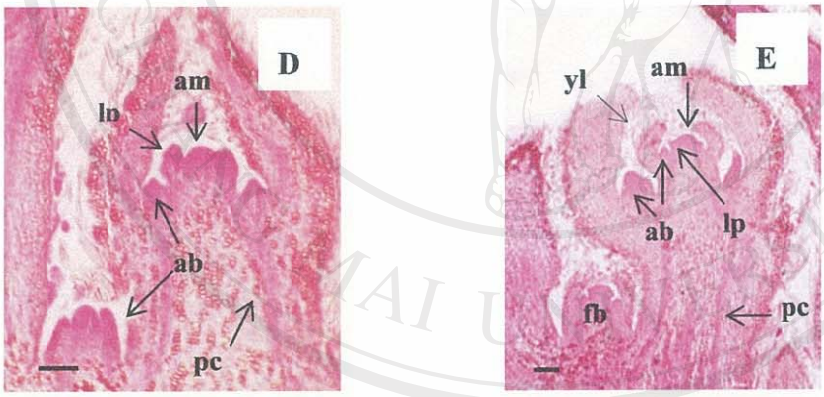



**Figure 6.3** Longitudinal section of terminal buds at vegetative stage at 38 days after flushing under warm temperature (A), and at day 35 of cold treatment (B), day 4 (C), day 11 (D) and day 18 after rising temperature of cold treatment (E) (ab = axillary bud, am = apical meristem, fb = inflorescence bud, lp = leaf primordial, pc = pro cambium, yl = young leaf, Scale = 0.2 mm)

### 6.3.3 Changes in physiological activities as affected by low temperature treatment

#### 6.3.3.1 Leaf total chlorophyll

As shown in Figure 6.4 total chlorophyll contents in litchi leaf at starting of the cold treatment (day 0) were around 350 mg chlorophyll $m^{-2}$ leaf. Leaf total chlorophyll tended to decrease with the longs duration of the experiment but no effected of temperature treatment on chlorophyll content of leaves were observed.

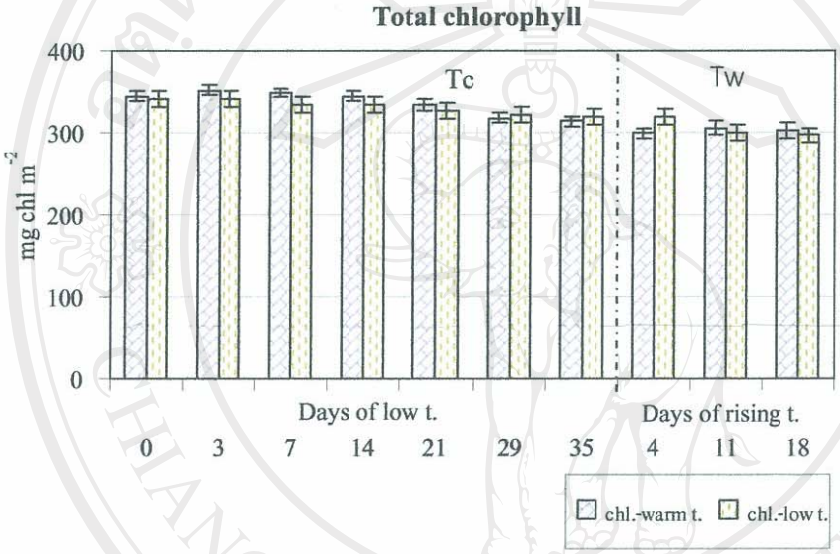



**Figure 6.4** Total chlorophyll concentrations in leaves under low temperature compared to warm temperature during two months

#### 6.3.3.2 Changes in photosynthetic rate, transpiration rate, stomatal conductance and leaf temperature

**1) Diurnal changes**

This experiment was designed on the basic methodology of difference in temperature treatments under the same light intensity. The results of these basic environmental conditions were confirmed by the similar diurnal pattern of all the treatment in parameters of *PAR* (relevant to light intensity) and leaf temperature. (Figure 6.5)

Diurnal changes of Pn, Tr, Gs, PAR and *Tl* as affected by low temperature treatment were measured at day 10, 17, 24 and 31 of cold treatment and at day 3 and 7 during temperature rising up period. Data were however shown only on day 10, 31 of cold treatment and on day 3

and 7 during temperature rising up period. The data of day 17 and 24 of cold treatment were ignored due to flushing of terminal bud of warm treatment, which supposed to affect sink strength and physiological activities of plant.

As shown in Figure 6.5, under Tc-period Pn, Tr and Gs of leaves under low temperature were significant lower than those under warm temperature treatment. On the day 24 of low temperature treatment, Pn- , Tr- and Gs-values were decreased drastically compared to the value on day 10 of the cold treatment.

At this measurement the studied plant were under flushing stage. In Tw-period, diurnal *Pn*, *Tr* and *Gs* remained constant low both for plants grown under warm and low temperature.

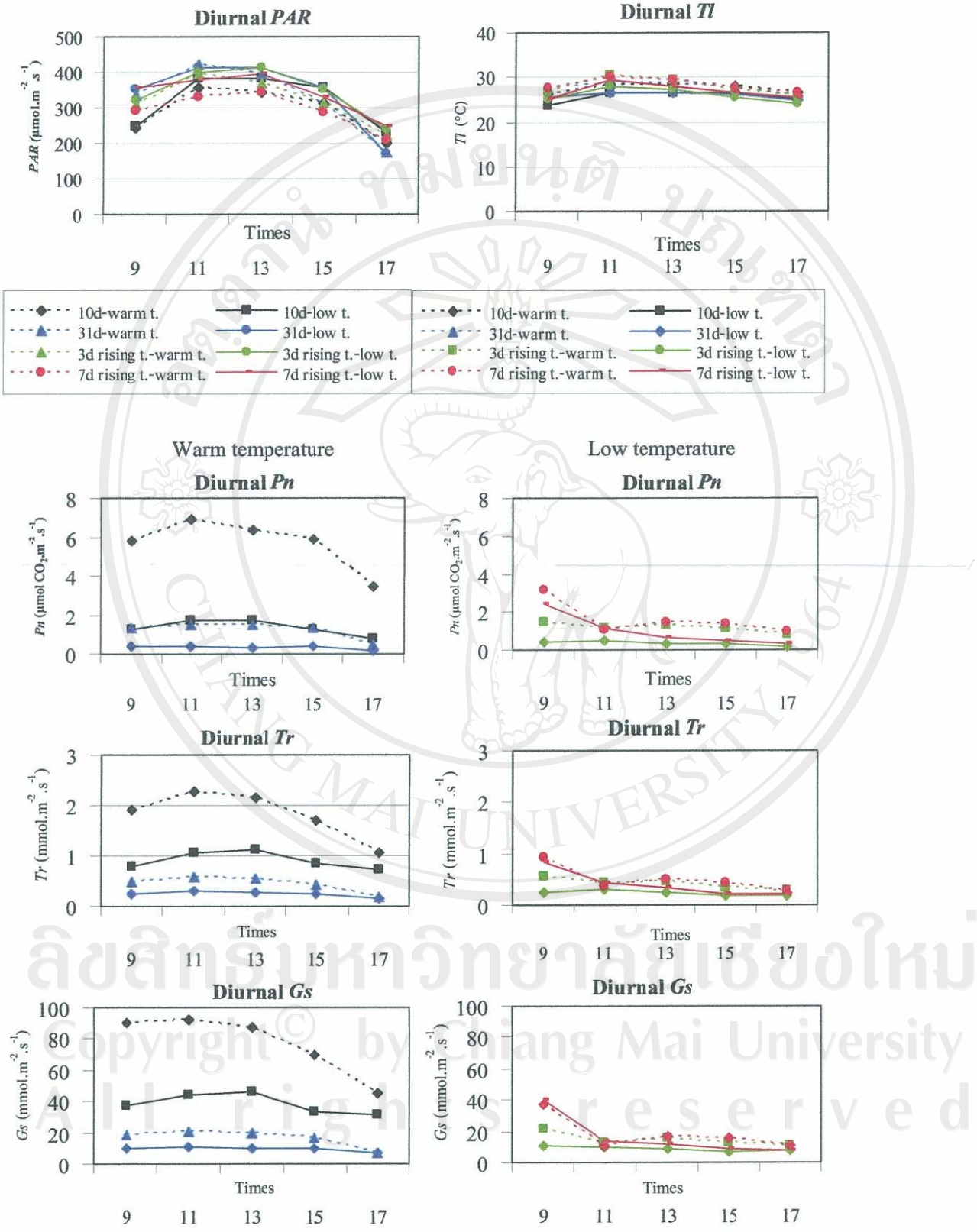



**Figure 6.5** Diurnal changes in *PAR*, *Fv/Fm*, *Pn*, *Tr*, *Gs* and *Tl* during treatment

**2) Photosynthetic activities changes**

In this experiment litchi trees were treated with low temperature regime (15/10°C) for 38 day and then exposed to stepwise temperature increase up to 29.5/24°C for another 18 days (Tc and Tw in Figure 6.6). Beside the *PAR* and *Tl* which unaffected by temperature treatment and remained relative constant throughout the experiment, *Pn* of litchi plant dropped drastically starting on day 21 of the treatment even under the warm temperature (37/27°C). Under warm temperature treatment, the average of normal value of *Pn* of litchi trees was 6 μmol $CO_2$ $m^{-2}$ $s^{-1}$, but dropped down to around 1.5 μmol $CO_2$ $m^{-2}$ $s^{-1}$. Whereas litchi leaves under low temperature treatment showed a fast response by decrease the *Pn* even within 7 days of the treatment, by which values of *Pn* decreased from 5 μmol $m^{-2}$ $s^{-1}$ and reached the lowest value of less then 1 μmol $m^{-2}$ $s^{-1}$ at day 21 of cold treatment. *Pn* of litchi leaves increased again when the temperature in growth chamber was rising up (Figure 6.6). Parallel patterns of physiological activities decrease were also detected by *Tr* and *Gs* both of warm and cold treatments (Figure 6.6).

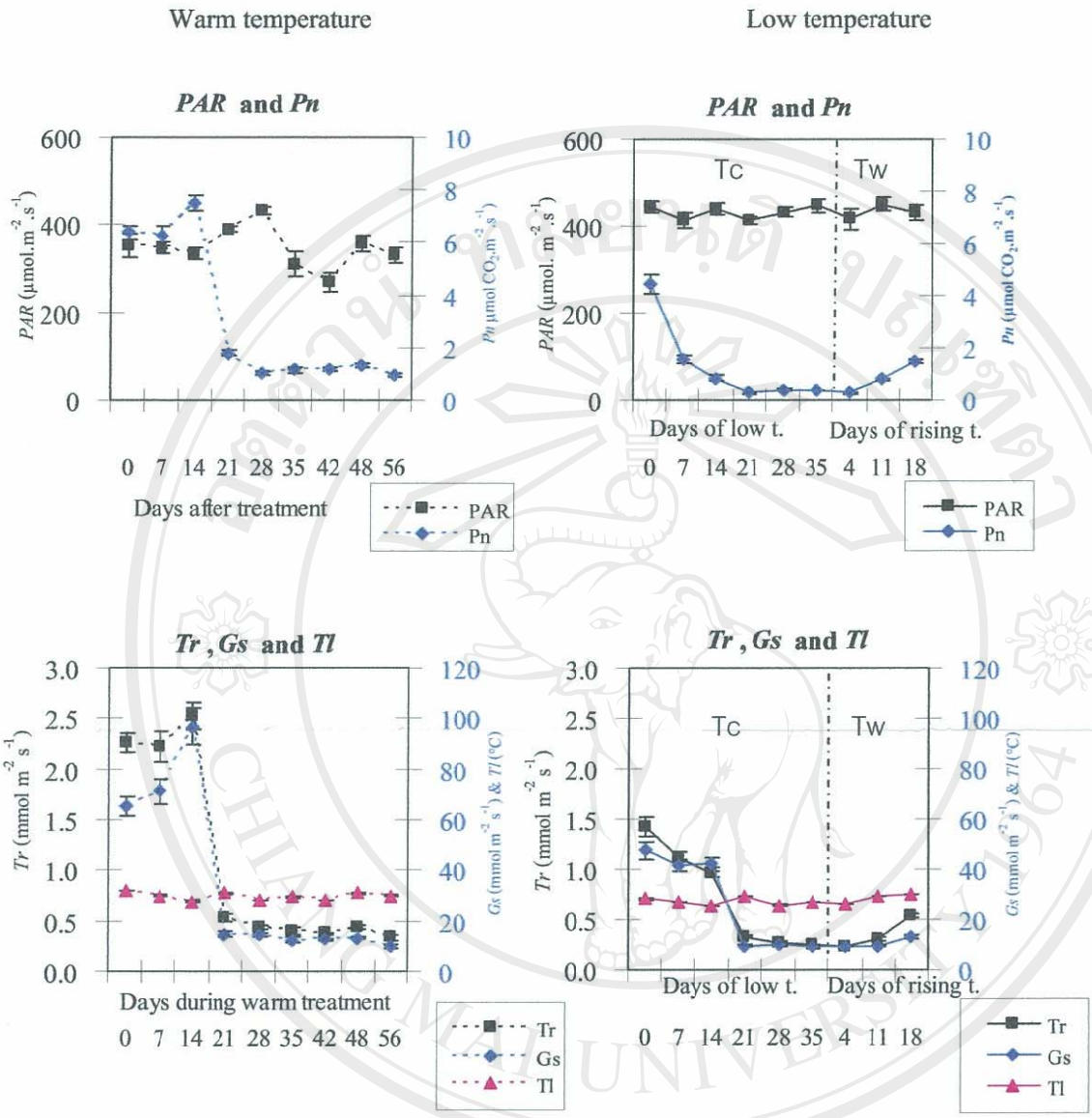



**Figure 6.6** Changes of *PAR*, *Fv/Fm*, *Pn*, *Tr*, *Gs* and *Tl* during the cold treatment and during temperature rising period



### 6.3.3.3 Change in assimilate content

Assimilate contents of leaves and roots were determined in the form of TNC and RS, comparing these of plants grown in warm and cold temperature. In the case of leaf, TNC and RS tended to increase consistently throughout the warm treatment. Under low temperature treatment TNC in leaves increased clearly during day 35 of cold period and day 4 of rising up the temperature then decreased at day 11 and 18 of rising up the temperature period (Figure 6.7). The similar trends were also found in TNC and RS of root.

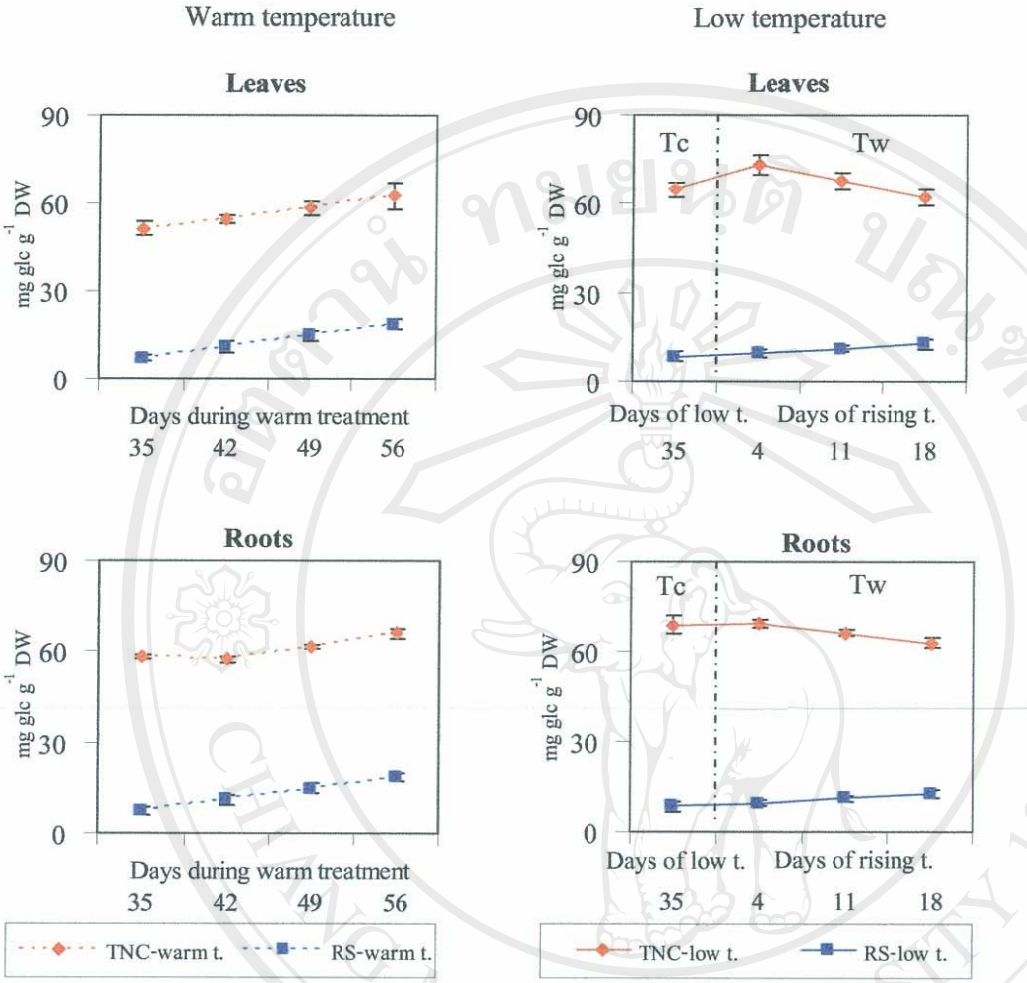



**Figure 6.7** TNC and RS concentrations in leaves and roots at warm and low temperature conditions during the second month of treatments

### 6.3.4. Effect of low temperate on hormonal level

Studies on the effect of low temperature on plant hormone were focused at the stage of bud transformation. In chapter 5, it was showed that bud development as affected by low temperature would be intensive after 28 days of cold exposure and under warming up temperature are 15/10°C. In this study the samples were therefore collected at the end of cold period (35 day of cold period) and at 4, 11 and 18 days after rising up the temperature. Comparing between buds from plant grown under warm and low temperature (Figure 6.8), it was found that under Tc-period Z/ZR and IAA concentrations were lower.

were lower. By transition from Tc-period to Tw-period, IAA and GA concentrations in buds stayed relative low, whereas Z/ZR concentrations in buds increased drastically from 17.9 ng $g^{-1}$ DW at day 35 of cold period to 84.9 ng $g^{-1}$ DW at day 18 of rising up the temperature period. Z/ZR concentrations increased significantly in leaves, bark, wood and xylem sap by rising up the temperature after cold period, whereas it did not increase in leaf diffusate. In leaf diffusate, IAA significantly increases under warm temperature treatment from 0.14 ng $leaf^{-1}$ (10 times increase). Under low temperature treatment, IAA leaf diffusate increase in small extent and stayed relative low only 0.36 ng $leaf^{-1}$ (1.5 times increase).

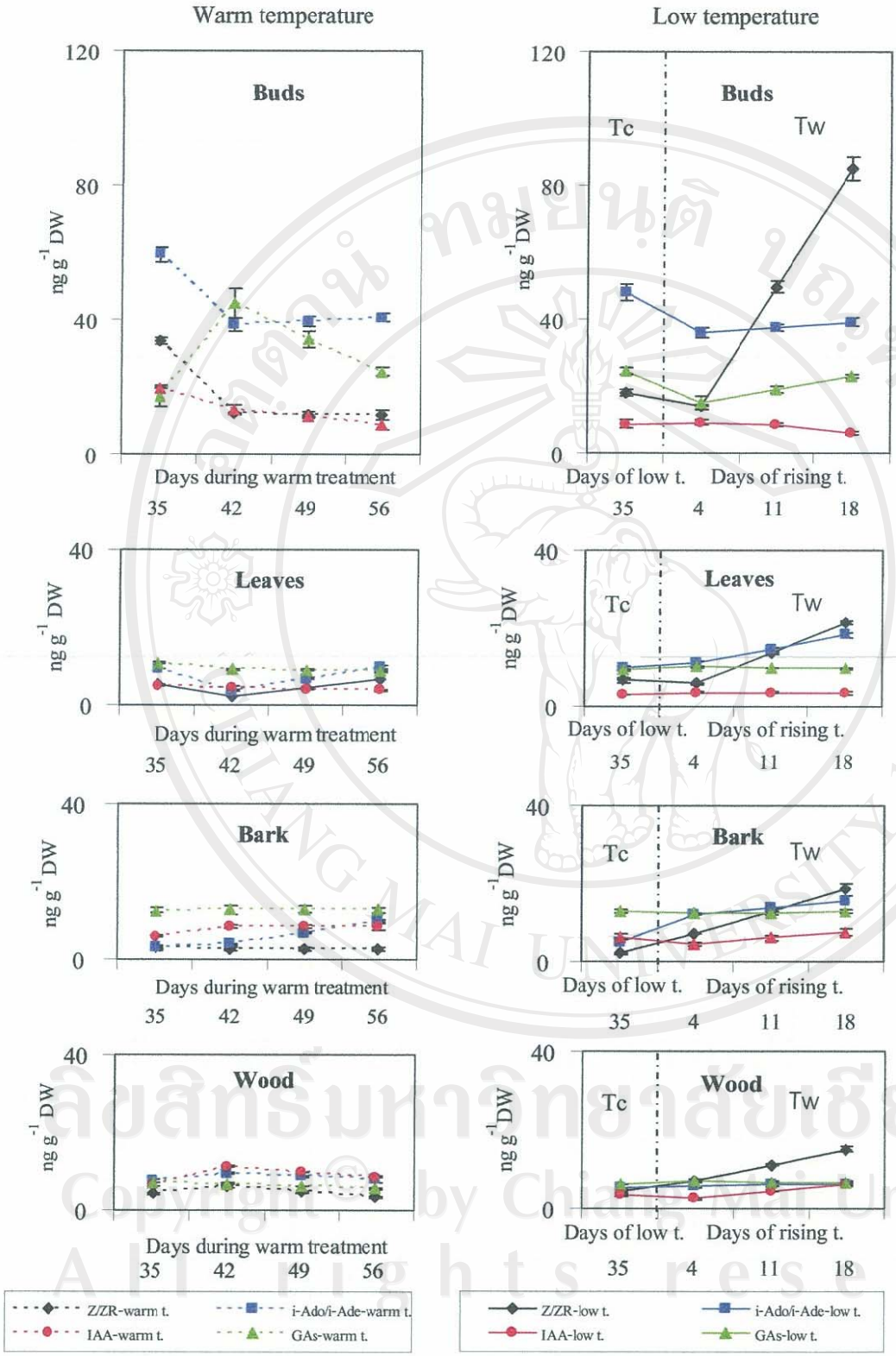



**Figure 6.8** Hormonal concentrations in plant tissues at warm and low temperature conditions during the second month of treatments

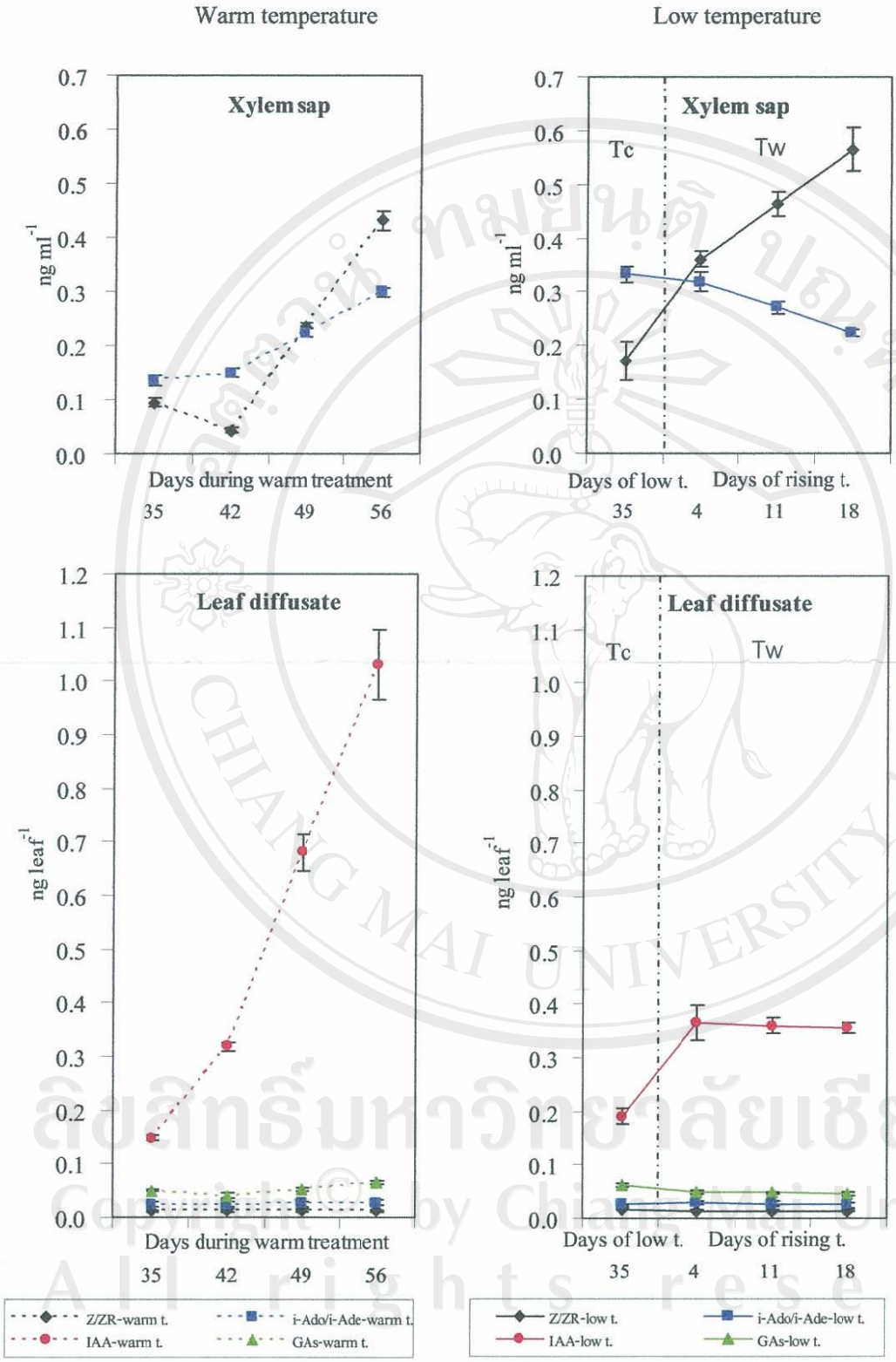



**Figure 6.8** (Continued)

**6.4 Discussion**

This experiment was designed to confirm the results from previous Chapters and to study physiological and biochemical changes of litchi tree at the transition period from low temperature treatment to warmer conditions. Most of the results confirmed the previous results and can be discussed as follows:

**6.4.1 Effect of low temperature on bud development**

According to the suitable temperature for promote flowering in litchi, the results confirmed an adequate condition of 15/10°C day/night temperature for 38 days duration. However, an appropriate warm temperature of 27-29/23-24°C seemed to be necessary to promote inflorescence bud development. In this study terminal buds stayed dormant at day 38 of cold treatment. By rising up the ambient temperature of the chamber, terminal bud started to develop at the apical meristem, procambium and leaf primordial. Full initiation of inflorescence bud could be firstly achieved within 17 days of warmer temperature after cold period. This confirmed the necessary of rising up the temperature after cold period to promote inflorescence bud formation. In this case, Batten and Lyndon (1990) also reported that in some species reversion of floral organs to leaf-like organs without affection their arrangement could be found by transferring plants from inductive to non-inductive conditions. Furthermore, shoot type can be reversed during morphogenesis by transferring trees warm-to-cool or cool-to-warm temperatures (Batten and McConchie, 1995; Nunez-Elisea *et al.*, 1996).

In litchi, flowering was marginal, especially in vigorous cultivars, with maximum of 25°C and minimum of 15°C, but satisfactory with cooler days or nights. Inflorescences could have a variable number of leaves and under some circumstances at temperatures above 25°C and floral buds might reverse to vegetative growth (Menzel and Simpson, 1994; Menzel, 2002b). After trees exposure in excessively high temperatures, during panicle differentiation would cause leaves on panicles to develop and flowers to atrophy (dwarf), especially when an induction phase was ceased with insufficient exposure to chill-inductive temperatures (Huang and Chen, 2003). However, Stern and Gazit (2003) proposed that under field conditions, full induction may occur under somewhat higher temperatures relative to those needed under constant day/night temperature regimes. Nunez-Elisea *et al.* (1996) suggested that inflorescence primordial of

mango, forming at axillary meristems of preformed nodes, dedifferentiated apparently and regressed to a meristematic condition in response to a sudden shift of trees from cool to warm temperatures. An incipient inflorescence primordial, which initiated in low temperatures, inflorescence buds ceased differentiation upon transfer to warm temperatures near 30°C with bud differentiation progressing vegetative. In comparison, the more differentiated inflorescence primordial as stage 2 inflorescence buds were not affected by the shift from low to warm temperature regime.

In conclusion, it can be emphasized that both steady low temperature (15/10°C) followed by a steady warming up of temperature to be 27-29/23-24°C are necessary to promote flowering in litchi tree.

**6.4.2 Effect of transition from cold-to-warm temperature on physiological activities**

Only low temperature without additional effect of different light intensity influences on photosynthesis, transpiration, stomatal movement and total chlorophyll content was studied. Litchi plants were cultivated under growth chamber condition, for similar light intensity, by content the ambient temperature regimes for 15/10°C and 35/27°C for low temperature and warm treatment, respectively. The results firstly confirmed the finding in previous experiment of chapter 3 and 5. Low temperature decreased clearly the values of *Pn*, *Tr* and *Gs*, but not affected the total chlorophyll content of the leaf.

Increase in TNC in leaves under low temperature was supported to be the major factor to decrease water potential in stomata and also affected the photosynthetic ability of relating enzyme, stomata then closed or opened less than usual. Gas exchange and vapour rate were then diminished.

How the TNC increased in leaves under low temperature but it did not increase in roots, was not yet fully explained. Low night temperature reduced the carbohydrate concentrations suggested mobilization from leaves. In this study TNC concentrations in terminal buds were not analyzed, but it could be expected to be at low level. Slow assimilate transportation out with low concentrations to sink organ due to low mobility under low temperature is the main reason (Paul *et al.*, 1992). With the limitation of carbohydrate supply and with less carbohydrate reserve, the terminal bud, therefore require a warmer temperature to promote a full development

of the inflorescence. Under only low temperature, floral induction may be promoted through an appropriate proportion of auxin and cytokinins (Naphrom *et al.*, 2004; Naphrom, 2004), which also confirmed in this study. Increase of ambient temperature promoted a higher rate of *Pn* and also the mobilization of carbohydrate to sink organ, which concurrently promote reproductive bud development. These finding also found in longan (Pichakum *et al.*, 2003; Kiatsakun 2004), litchi (Koo-Duang, 1984; Menzel *et al.*, 1995; Hieke, 2000; Thonglem, 2000) and other species (Goldschmidt *et al.*, 1985; Goldshmidt, 1999).

### 6.4.3 Relation between hormonal change and reproductive bud development during transition from cold-to-warm temperature

Increase ambient temperature could increase the cytokinins (Z/ZR) level in bud on day 11-18 of rising up the temperature. Auxin however decreased in bud, with an increase in small extent in leaves at the same studied period, accompanied by inflorescence bud formation. The same result also found in the previous chapter. High concentrations of CKs during floral bud formation were similar to longan (Hegele *et al.*, 2004; Chen *et al.* (1997), mango (Naphrom, 2004; Naphrom *et al.*, 2004) and litchi (Chen, 1990, 1991; Stern *et al.*, 2003; O'Hare, 2004). Moreover, Chen *et al.* (1997) reported that during floral initiation in shoot a large decrease in CK glucosides and an increase in Z and ZR activities in buds were observed, which was higher than CK concentrations at the leaf flush stage. In addition, low concentrations of auxin in bud were also support Bernier *et al.* (1993) and Hegele *et al.* (2004). Balance of cytokinin/auxin was reported elsewhere to have a close correlation of flower bud development (Chen 1991; Hegele *et al.*, 2004; Naphrom, 2004; Naphrom *et al.*, 2004). Cytokinin and auxin interaction was also reported, which affected by cytokinin accumulation in plant organs (Bangerth, 1994, 1997; Bangerth *et al.*, 2000). In this experiment, transition of temperature from low to warm increase only 4-folds of auxin in leaf diffusate. This phenomenon occurred at day 11-18 of warmer temperature treatment, which suggested an effect of cold-to-warm treatment on inflorescence development through effect of cytokinin/auxin balance.

**6.5 Conclusion**

1) Appropriate temperature requires to promote flowering in litchi as 'Hong Huay' depended on two keywords ; adequate low temperature of 15/10°C for a period of 38 days, and adequate transitioning warm temperature of 27-29/23-24°C for at least 11 days.

2) Development of reproductive bud was firstly occurred in tissue level by flattening of apical meristem and appearance of leaf primordial, which already took place within 4 days of warmer temperature treatment, and can be called as "floral differentiation". The whole period of floral differentiation could be last for 2 weeks to complete the inflorescence development at terminal bud and in axillary bud. Only at this stage the swelling would be firstly observed.

3) Cks must be high, whereas IAA content proportionally low at the time of floral initiation and GAs normally stayed at the low level.