Chapter 5

Development of Terminal Bud and Changes in Physiology and Biochemistry of Litchi Plant Grown Under Low Temperature

5.1 Introduction

As shown in chapter 3, low temperature at 15/10°C diurnal cycle did affect many physiological changes such as photosynthesis and transpiration, assimilate accumulation and endogenous hormonal content in 'Hong Huay' litchi trees. It also induced flower bud formation. Moreover, the 38 days of cool period was more effective than 28 days of cool temperature duration in promote floral induction. In addition, the study in chapter 4 indicated that exported of IAA from leaves varied depending on the age of leaf. Young leaves had greater diffusible IAA concentration than old leaves. These preliminary results suggested the first information that cold temperature promote floral induction in litchi through its effect on physiological and biochemical changes, i.e. hormonal balance, in which Z/ZR and IAA supposed to play a significant role. Increase of IAA level in flushing shoot short prior to flowering inhibit the floral development. These finding must be confirmed by the parallel study of bud morphology (bud section) and physiology and biochemistry.

In general, shoot apical meristem initially gives rise to vegetative organs such as leaves. However, under some conditions i.e. through appropiated environmental and endogenous signals, the SAM makes transition to reproductive development and production of flowers (Bernier, 1988; Levy and Dean, 1998; Fletcher, 2002). Certainly, an inflorescence morphogenesis required presence of leaves during active bud growth such as in mango (Nunez-Elisea *et al.*, 1996) and longan (Sruamsiri *et al.*, 2003).

Leaves take on critical role influencing response of plants to environment. Furthermore, photoperiod and irradiance are perceived mainly by mature leaves in intact plants. Temperature is perceived by all plant parts, whereas vernalization is perceived by root system. Actually, signals are generally transported from leaves to apical meristem in phloem with assimilates. On the other hand, the signals originating in roots are presumably transmitted in xylem with

transpiration stream (Bernier *et al.*, 1981). Thus, under low temperature, leaves and roots are important parts of tree for floral stimulus synthesis, and terminal bud is certainly the important target of signal to trigger the development.

For floral induction, a model of multifactorial control was proposed that a number of promoters and inhibitors, including phytohormones and assimilates, were involved in controlling the developmental transition. Moreover, different factors could be restricting for flowering in different genetic backgrounds and/or under particular environmental conditions (Levy and Dean, 1998). Normally, auxin affects many developmental processes such as induction of cell division, stem and coleoptile elongation, apical dominance, induction of rooting, and vascular tissue differentiation. The physiological responses are also affected from GA including changes in juvenility, flowering and floral development, promotion of fruit set, fruit growth, and seed germination (Srivastava, 2002; Taiz and Zeiger, 1998). Furthermore, CKs changes in association with plant development have been reported (Dewitte *et al.*, 1999; Howell *et al.*, 2003). In agricultural practices flowering of several fruit trees can be induced through the use of some chemical substances. PBZ, a gibberellin synthesis inhibitor, is widely applied in mango and durian orchards. In longan, $KClO_3$ is also well known to enhance flowering. However, with sofar achieved research results floral induction of litchi tree seems not consistently response to $KClO_3$ and/or PBZ or any chemicals.

Such CKs promoted flower bud differentiation in fruit species, for example, litchi (Chen, 1990, 1991), longan (Chen *et al.*, 1997; Hegele *et al.*, 2004) and Japanese pear (Ito *et al.*, 2001). Beside the plant growth regulators, sugars also play a regulatory role in floral induction, based on circumstantial evidence in mango (Rameshwar, 1989). Low temperature is proved to be more effective to promote flowering.

In this chapter, responses of litchi trees, especially terminal bud development as well as changes in physiological aspects and essential biochemical substances content during the first month of low temperature treatment will be investigated. This study period is expected, from chapter 3 results, to be a floral induction period, so that morphological change of terminal bud as affected by cold treatment will be parallelly studied.

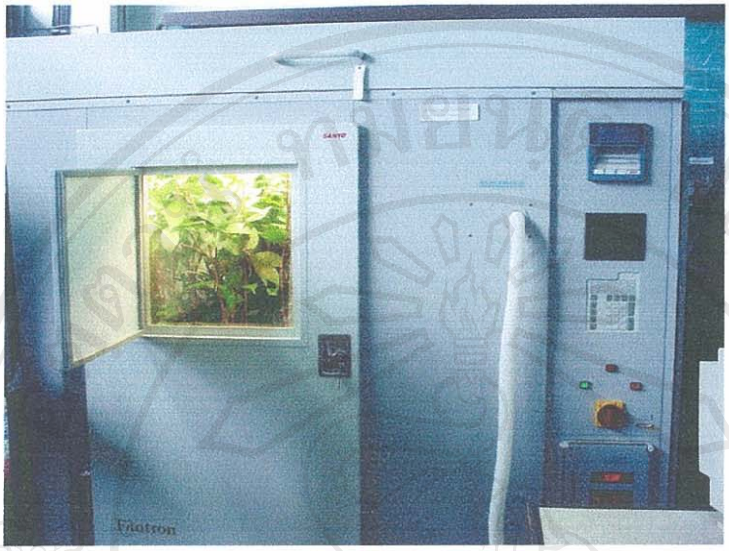
5.2 Material and methods

5.2.1 Plant sample and cold treatment

These experiments were determined on one year-old air-layered 'Hong Huay' litchi trees growth in plastic bags with 75 day-old mature leaves on terminal shoots. Plants were divided into two treatments. The first treatment of sixty trees were kept for 45 days in the growth chambers (Fitotron SGC970, Sanyo) to experience a diurnal day/night temperature regime of 15/10°C and 12 h day/night photoperiod. After 45 days of cold treatment, the temperature in growth chamber was stepwise increased to be 27.5/17.5°C 39 days for observation of flowering (Figure 5.2). The second treatment, plants were kept under warm temperature in the greenhouse which the relative humidity was controlled with water automatic sprayer for 15 minutes interval during 11:00- 17:00 pm. (Figure 5.3). The experiments were conducted during October 2002 to March 2003 at Lampang Agricultural Research and Training Centre, Rajamangala Institute of Technology, Thailand.

As shown in Figure 5.2 and 5.3, the temperatures in growth chamber (cold treatment) during 45 days of cool period were relative constant at 15/10°C day/night time. Time required to rising the temperature to be 27/17.5°C day/night was 15 days, before kept constant presumably to promote flowering. Maximum and minimum temperatures in greenhouse were around 40-45°C and 35-38°C at noon and night time, respectively. Temperature difference between warm- and cold treatment was average 25-30°C. In consideration to RH, in growth chamber diurnal RH was kept constant during 65-75% (data not showed), whereas in greenhouse RH also remained relative constant at 70% to 75%.

A. Growth chamber



B. Greenhouse

Figure 5.1 Growth chamber (A) and greenhouse (B) as cold and warm temperature treatments, respectively

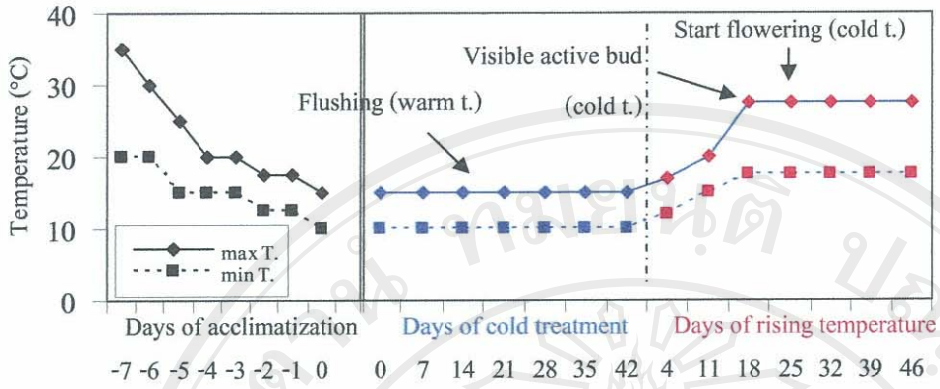


Figure 5.2 Maximum and minimum of ambient temperature in the growth chamber during the acclimatization period, cold treatment, and temperature increasing period (flowering promoting period)

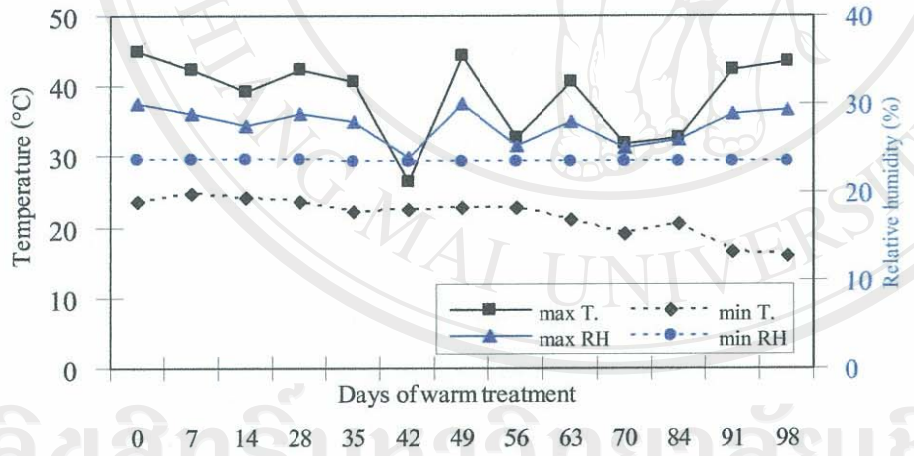


Figure 5.3 Maximum and minimum temperatures and relative humidity in greenhouse kept under natural conditions during the experimental period

5.2.2 Data collection

5.2.2.1 Morphological change of the terminal buds

Determination of the developmental stage of the terminal buds was done by using paraffin embedded technique and method described by Sujawipun (1982). Five shoot tips, with 0.4-0.7 cm length, per treatment were sampling at day 7, 14, 21 and 28 of cold treatment. The shoot tip samples were stopped activity and fixed with 70 % FAA (formalin-acetic acid alcohol; 18 of 70% ethyl alcohol: 1 glacial acetic acid: 1 formalin) about 1 to 2 weeks. Then they were suctioned by vacuum pump at 600 mm Hg for 1 h and left under vacuum condition for at least 24 h until bubble was not occur. After that the shoot tip samples were dehydrated for 24 h in a solution containing of tertiary butyl alcohol (TBA) mixed with 5 series of 50, 70, 85, 95 and 100% alcohol respectively. By TBA plus alcohol 100% erythrosine was added as dye. Samples were then infiltrated 3 times with pure TBA for 12 h each then transferred into solution of pure TBA mixed with paraffin oil (1:1), and pure paraffin oil for each 12 h respectively. After that the samples were kept in paraplast solution in the oven (55-60°C) around 2-3 months and embedded in paraplast. Thus, the paraplast samples were sectioned at thickness of 10 μm using rotary microtome, and affixed on the slide with 2% Hapt's adhesive. The slides of microtome section were studied and photographed under stereo microscopy.

5.2.2.2 Percentage of flowering

Ten litchi trees (replications) were sampled for data collecting. The collected data were the percentage and days to visible active buds, flowers and flushes similar to chapter 3.

5.2.2.3 Changes in leaf physiological aspects

The physiological change of leaves as affected by low temperature was studied through the following parameters: chlorophyll content, *Fv/Fm*, *PAR*, *Pn*, *Tr*, *Gs* and *Tl*. Plant efficiency analyzer (Model PEA, Hansatech Instruments, U.K.) was used to measure *Fv/Fm* and portable photosynthesis system (Model CIRAS-1, PP System, U.K.) used for measuring *PAR*, *Pn*, *Tr*, *Gs* and *Tl* change.

The measurement took place on eighteen fully expanded mature leaves (18 replications). The third leaves from shoot apexes were randomly selected for the measurements. The last couple of leaflet from the basal past were used one side for *Fv/Fm* measurement, and the opposite side leaflet for chlorophyll meter (chlorophyll content); and CIRAS-1 PP System measurement, respectively.

Plant efficiency analyzer were carried out on the middle leaf surface during 10:30 to 11:00 o'clock, whereas the measuring of CIRAS-1 PP System was carried out during 10:30 to 12:00 o'clock as in chapter 3.

1) Measurement of total chlorophyll content

The same leaves as photosynthetic rate measurement were investigated during 11:00-11:20 o'clock using chlorophyll meter (Model SPAD-502, Minolta, Japan), an instrument for determine foliar leaf chlorophyll concentration *in vivo*. A calibration curve of the SPAD value was made based on the chlorophyll extraction method.

To produce a calibration curve, two fully matured leaves were randomly selected per one terminal shoot. One leaflet from each leaf was used and combined the value together as one sample. The measurement was made on 36 samples (72 leaflets). On each leaflet measurement of SPAD value was made at 4 positions on mid-region of leaf blade. The four values were then averaged to obtain a single SPAD value from each leaflet. For chlorophyll extraction four leaf disks per leaflet were punched out from the leaf blade at the same area with chlorophyll SPAD measurement using 0.3 cm^2 paper punch. Chlorophyll was eluted from these disks by dipping in 10 ml 80% acetone. Samples were stored in the dark at room temperature for 48 h. Absorbance readings were obtained by using Jenway 6300 Spectrophotometer at 664 and 642 nm in 1 cm cuvette, and chlorophyll concentration was calculated according to the following formula and expressed as mg m^2 of leaf:

$$\text{Total chlorophyll (mg m}^{-2}) = (7.12\ A664 + 16.8\ A642)\ [n\pi r^2]$$

The procedure was describes by Marini and Marini (1983) and modified by Schaffer and Gaye (1989) and Kaosampun (1997).

5.2.2.4 Changes in endogenous hormonal contents in plant tissues

One leaf each from twenty trees per treatment was sample for hormone analysis. Samples were collected 5 times at 3, 7, 14, 21 and 28 days during cold treatment. Leaf samples, buds, bark, wood and xylem sap were immediately frozen in liquid nitrogen and freeze dried before stored at -20°C until analyzed, whereas the leaf diffusate samples were kept directly under -20°c. Three groups of hormones; CKs (Z/ZR, i-Ado/i-Ade), IAA and $GA_{1,3,20}$, were determined at the University of Hohenheim, Germany using the RIA method as described in chapter 3.

5.2.2.5 Changes in carbohydrate contents in leaves and roots

Similar to the determination of TNC and RS described in chapter 3, twelve leaves were randomly sampled from the leaves used for leaf diffusate collection. Root sample were made from five trees per treatment. The leaves and roots samples were collected 5 times at 3, 7, 14, 21 and 28 days of the cold treatment. Analyzing procedures TNC and RS were made at Faculty of Agriculture, Chiang Mai University using the same methods as described in chapter 3.

5.3 Results

5.3.1 Effect of temperature regime on flushing or flowering

As shown in Table 5.1, plants grown in greenhouse produced no flower but flushing. Within 14 days of the experiment (October 29, 2002) litchi trees started flushing. Under greenhouse condition 70% of the plants produced young leaves, whereas the other 30% remained dormant. In the low temperature treatment, plants remained dormant during 45 days of treatment, but they became active to reach visible active bud stage within 18 days after rising up the temperature. Up to 80% of the buds became active within 25 days of warming up the ambient temperature to be 27.5/17.5°C and at this stage some shoot started flowering.

Table 5.1 Effects of temperatures on leaf flushing and flowering

(10 replications)

Parameters	Low temperature treatment	Warm temperature treatment
Days to start flushing (days)	0 b	14 a
Percentage of flushing shoots (%)	0 b	70 a
Days to visible active bud (days)	18 a	0 b
Percentage of active buds (%)	80 a	0 b
Days to start flowering (days)	24 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.

5.3.2. Development of the terminal buds under low temperature

Developmental stage of terminal bud were determined at 1, 2, 3 an 4 weeks of low temperature treatment using paraffin embedded method. The results revealed that apical meristem of litchi held in warm temperature was a spherical in shape of dome and had only leaf primordial as vegetative phase during the measuring period (Figure 5.4 A). Under low temperature regime, meristem of dormant apical bud was also spherical shape accompanied by leaf primordial at its base during 1-3 weeks of cold treatment (Figure 5.4 B-D). However, at the 4th week of the treatment, apical meristem seemed to slightly extend and synchronized with the initiation of meristem of axillary bud (Figure 5.4 E).

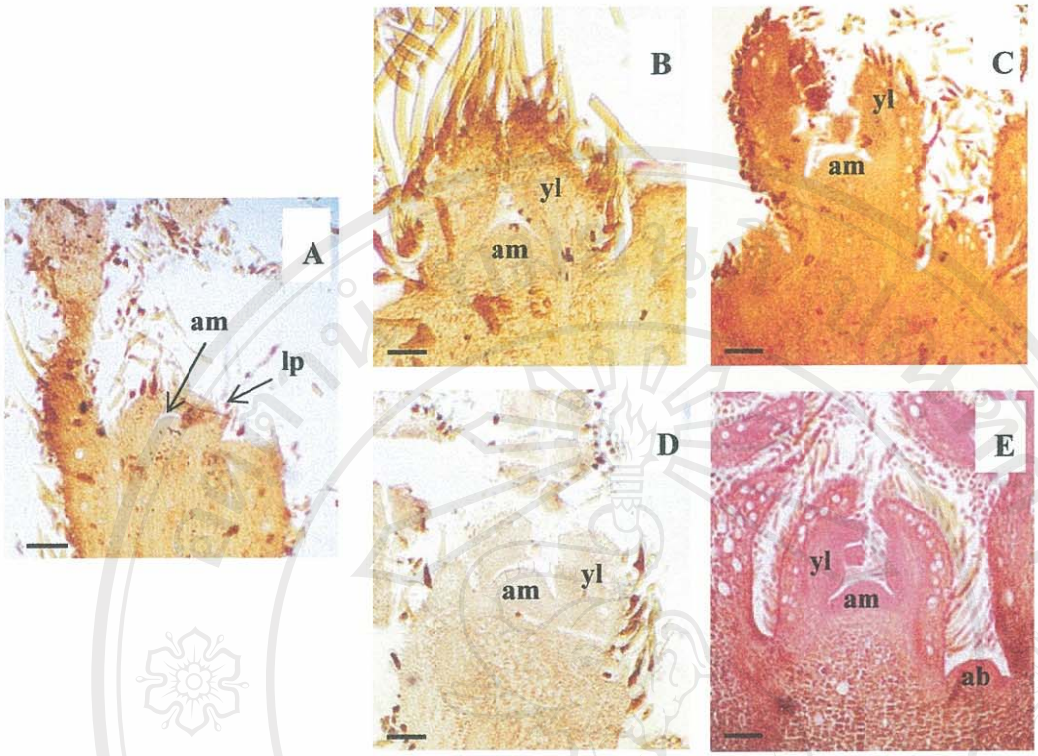


Figure 5.4 Longitudinal sections of the terminal buds at vegetative stage at 1st week of warm temperature treatment (A), and developmental stage of terminal buds at 1st week (B), 2nd week (C), 3rd week (D) and 4th week (E) of low temperature treatment (ab = axillary bud, am = apical meristem, lp = leaf primordial, yl = young leaf, Scale = 0.2 mm)

5.3.3 Change in total chlorophyll content

Total chlorophyll concentrations of intact leaves were measured by using chlorophyll SPAD meter and readjusted the value with calibrating curve achieving from total chlorophyll analysis. The results revealed that during 42 days of cold treatment leaf total chlorophyll content remained constant and not significant difference in leaf between warm and low temperature treatments. Total chlorophyll concentration of litchi was approximately 280 mg chlorophyll m^{-2} leaf (Figure 5.5).

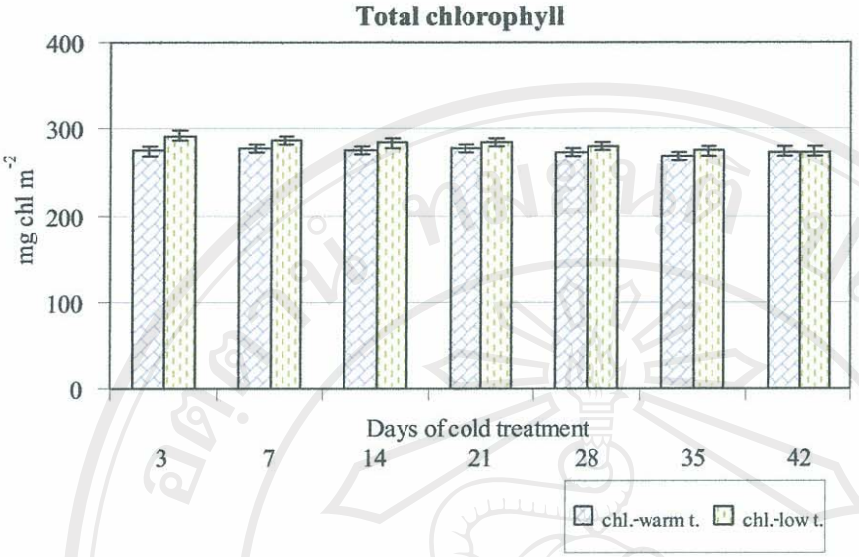


Figure 5.5 Total chlorophyll concentrations in leaves at warm and low temperature conditions during 42 days of treatments

5.3.4 Effect of low temperature on physiological activities

5.3.4.1 Diurnal change of photosynthesis, transpiration and stomatal movement

As shown in Figure 5.6 *PAR* of warm condition in a greenhouse increased from 8:30 am to arrive the maximum of 635 µmol m^{-2} s^{-1} at 10:30 am and decreased in the afternoon. Parallel to *PAR*, *Pn* of litchi leaves also gradually increase in the morning. *Pn* values reached its peak of 3.3 µmol CO_2 m^{-2} s^{-1} at 14:30 pm. In growth chamber, *PAR* was a maximum at 297 µmol m^{-2} s^{-1}, which was 53% lower than that of greenhouse condition. *Pn* of the litchi leaves remained constant whole day at around 0.4 µmol CO_2 m^{-2} s^{-1}, which was only 12.12% of *Pn* of plant grown in greenhouse.

Tr and *Gs* were also decreased under low temperature regime in growth chamber to those of greenhouse. Under low temperature, transpiration rate of litchi leaves at 12:30 pm was around 41.67% of under greenhouse condition, whereas *Gs* value was only 24% under low temperature compared to that under warm temperature. On this result, low temperature seemed to affect stomatal movement stronger than transpiration rate.

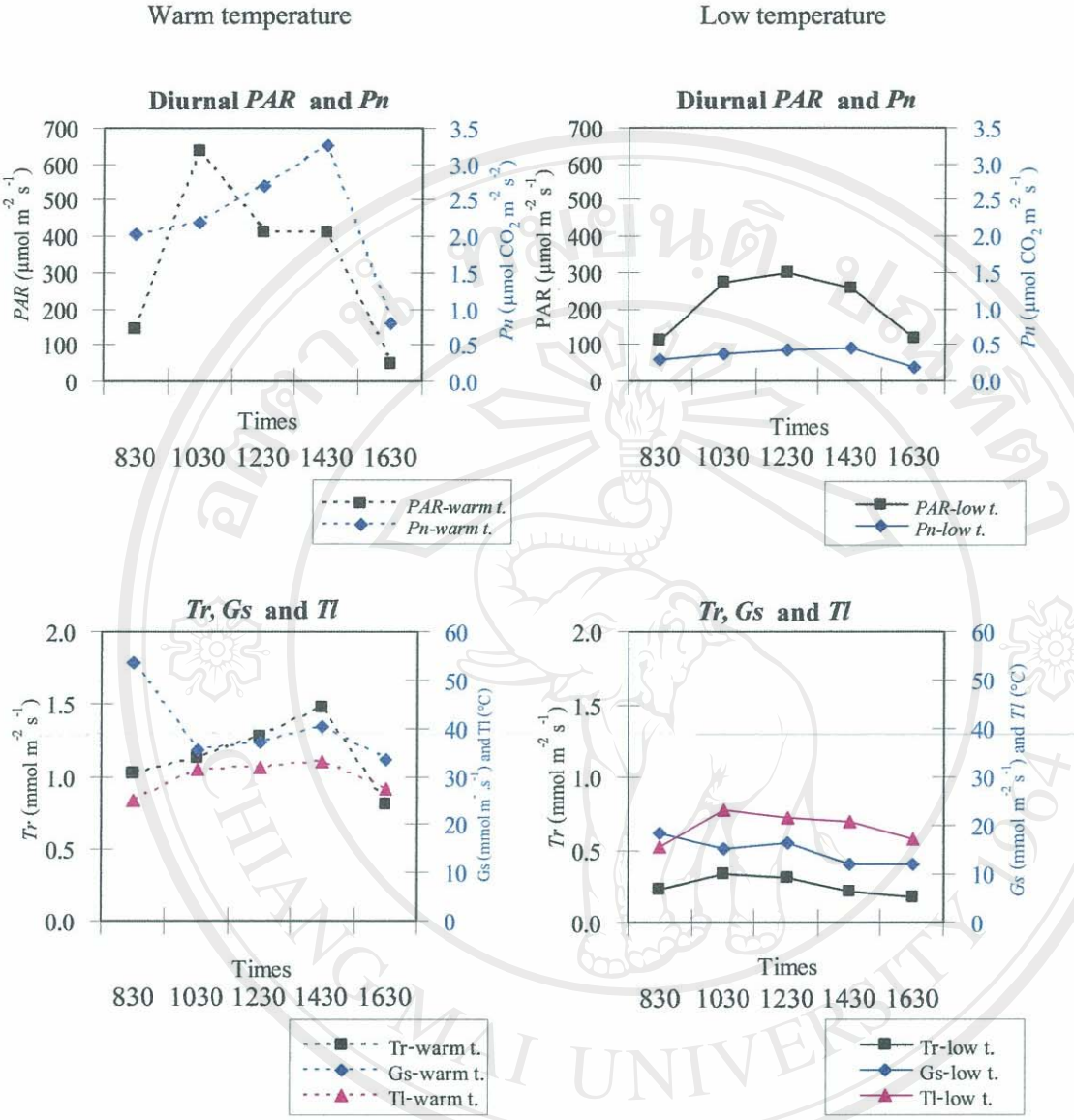


Figure 5.6 Diurnal changes in *PAR*, *Fv/Fm*, *Pn*, *Tr*, *Gs* and *Tl* at day 24 of treatments

5.3.4.2 Change of physiological activities during cold treatment and during rising up the temperature regime

In this experiment, plants were kept under low temperature regime (15/10°C day/night) for 45 days (Tc) and after that the ambient temperature was rising up to arrived 27.5/17.5°C (Tw). The changes of temperature regime were followed the natural temperature of winter condition of highland in northern Thailand, for which low temperature was extended from November to

January followed by temperature increment in February. Litchi in northern Thailand started flowering in January.

To study the effect of temperature regimes on physiological activities, all the measured values of *Pn*, *Fv/Fm*, *Tr*, *Gs* and *Tl* at 10:30-12:00 am were compared. Under greenhouse conditions, *PAR* and *Pn* values fluctuated due to unstable weather (sun shine/cloudy sky) in October, very strong. The same fluctuation was also observed on transpiration and stomatal movement. The relative stable values were achieved from *Fv/Fm* and *Tl* which depended on measurement methodology (*Fv/Fm*) and adaptation efficiency of leaf to maintain its appropriate temperature in the case of *Tl*.

In growth chamber, *PAR* values fluctuated around 400 μmol m^{-2} s^{-1} throughout the experiment, whereas physiological change according to temperature regimes could be detected on *Pn*, *Fv/Fm*. In both cases, the values of *Pn* fluctuated at around 1 μmol CO_2 m^{-2} s^{-1} (18% of greenhouse condition) under low temperature but increased to 3 μmol CO_2 m^{-2} s^{-1} under warm temperature regime of 27.5/17.5°C. A slight increase of *Tr*, *Gs* and *Tl* also observed in Tw-period compared to Tc-period, although the values were much lower than those of plants grown under greenhouse condition (Figure 5.7).

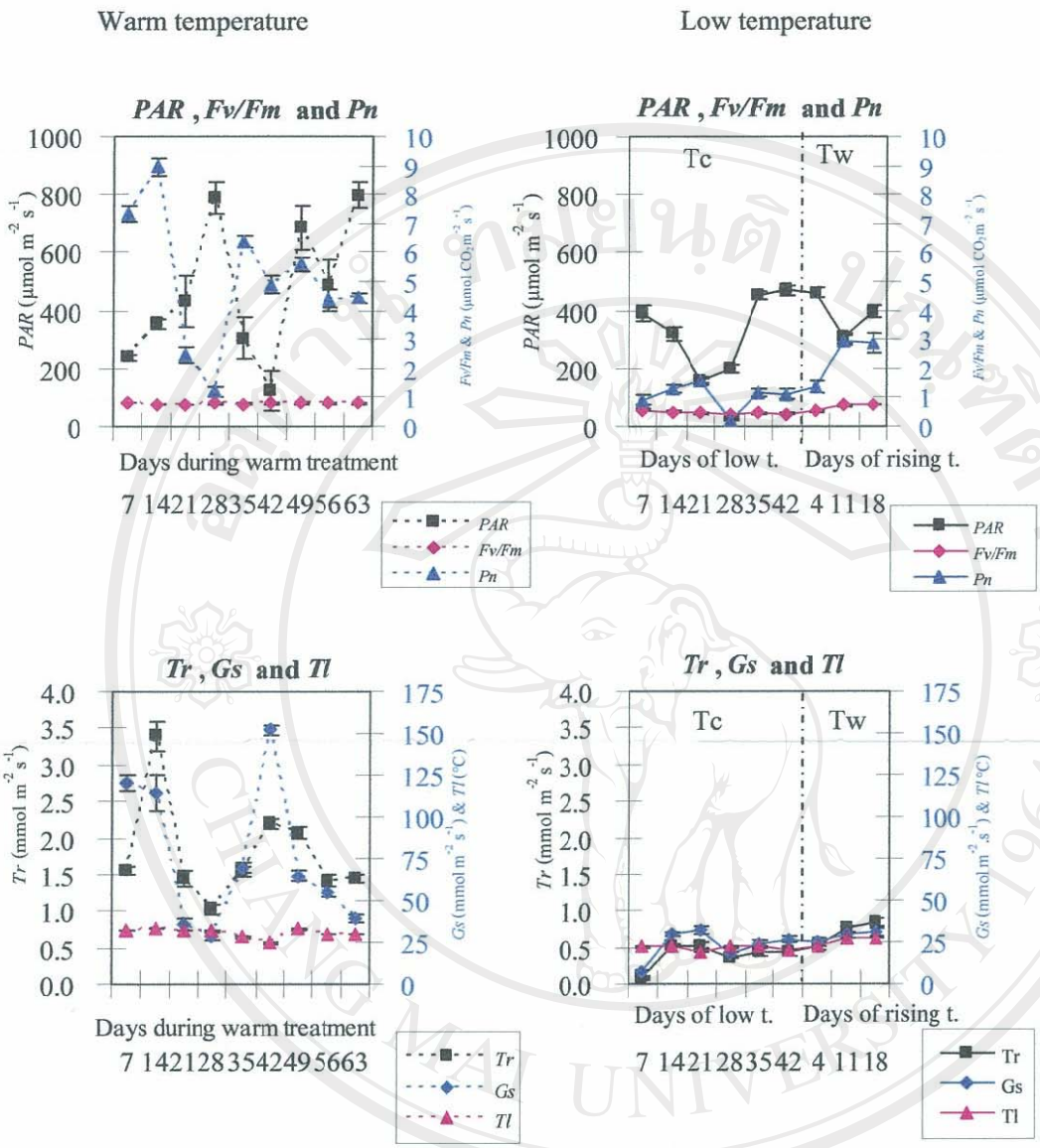


Figure 5.7 Change of *PAR*, *Fv/Fm*, *Pn*, *Tr*, *Gs* and *Tl* during the cold treatment and during temperature rising period

5.3.5 Change of carbohydrate content in leaves and root under low temperature

Carbohydrate content in litchi leaves and roots were analyzed for mg glucose g^{-1} DW of plant tissues. In general, in warm temperature condition litchi leaves and roots contained similar amount of TNC concentrations at 41.9-53.5 mg glucose g^{-1} DW and amount of RS at 10.8-21.3 mg glucose g^{-1} DW (Figure 5.8). Under low temperature treatment, TNC in leaves increased to

be more than 60 mg glucose g^{-1} DW, whereas no effects were detected on TNC in roots as well as RS in leaves and root.

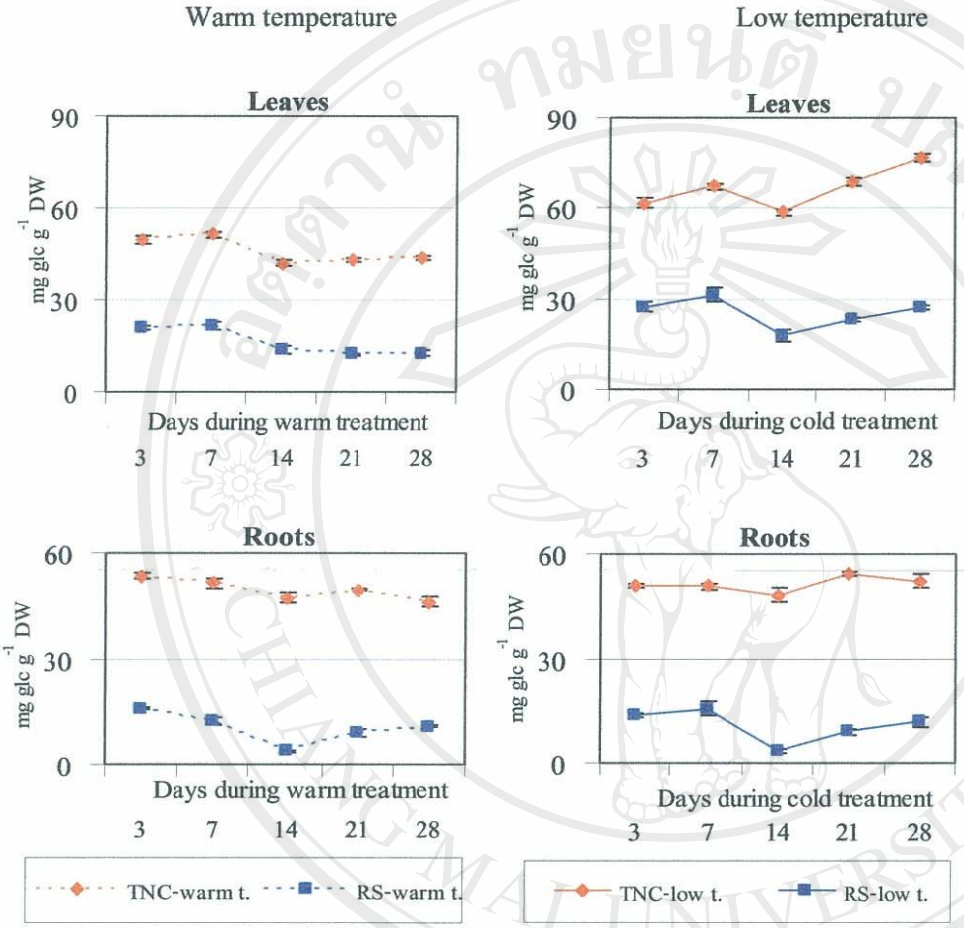


Figure 5.8 TNC and RS concentrations in leaves and roots at warm and low temperature conditions during the first month of treatments

5.3.6 Change of endogenous hormonal content under low temperature condition

Levels of cytokinins (Z/ZR and i-Ado/i-Ade), GAs ($GA_{1, 3, 20}$) and auxin (IAA) in terminal buds, leaves, bark, wood, xylem sap as well as leaf diffusate were determined in litchi plants grown in greenhouse as well as in growth chamber under cold temperature. The results are concluded and compared in Figure 5.9. Due to limiting space in growth chamber, analysis was done only during 28 days of cold treatment, although the treatment was longer for 45 days.

Under general warm temperature condition of greenhouse, hormone levels were the highest in buds, followed by barks, leaves and wood, respectively. Only in little amounts were found in xylem sap and leaf diffusate. Comparing from tissue by tissue, starting from buds, the dominant plant growth regulator found was GAs, which cold temperature had no significant effect on accumulating amount. In the opposition, buds under low temperature treatment had significant higher amount of IAA but lower level of Z/ZR.

In leaves, the same trend of hormone level was also detected, in which IAA concentrations increased but Z/ZR concentrations decreased as affected by low temperature treatment. GAs remained again the highest throughout the study period. GAs in bark, however, decreased in low temperature together with Z/ZR, whereas in this tissue low temperature still could not decrease the level of IAA.

Due to a very low level of growth regulators content in wood, low temperature showed no effects on hormone concentrations, even in the case of i-Ade/i-Ade. In xylem sap, which very close related to hormone concentrations produced in root and translocate *via* the transpiration stream to the upper plant parts, it was found that cold temperature decreased the content of Z/ZR but not i-Ado/i-Ade. In leaf diffusate, only the IAA was detected to be fluctuated with increasing trend in low temperature treatment. The other plant growth regulators were similar to those tissues from greenhouse both in the amount and trend during cold treatment.

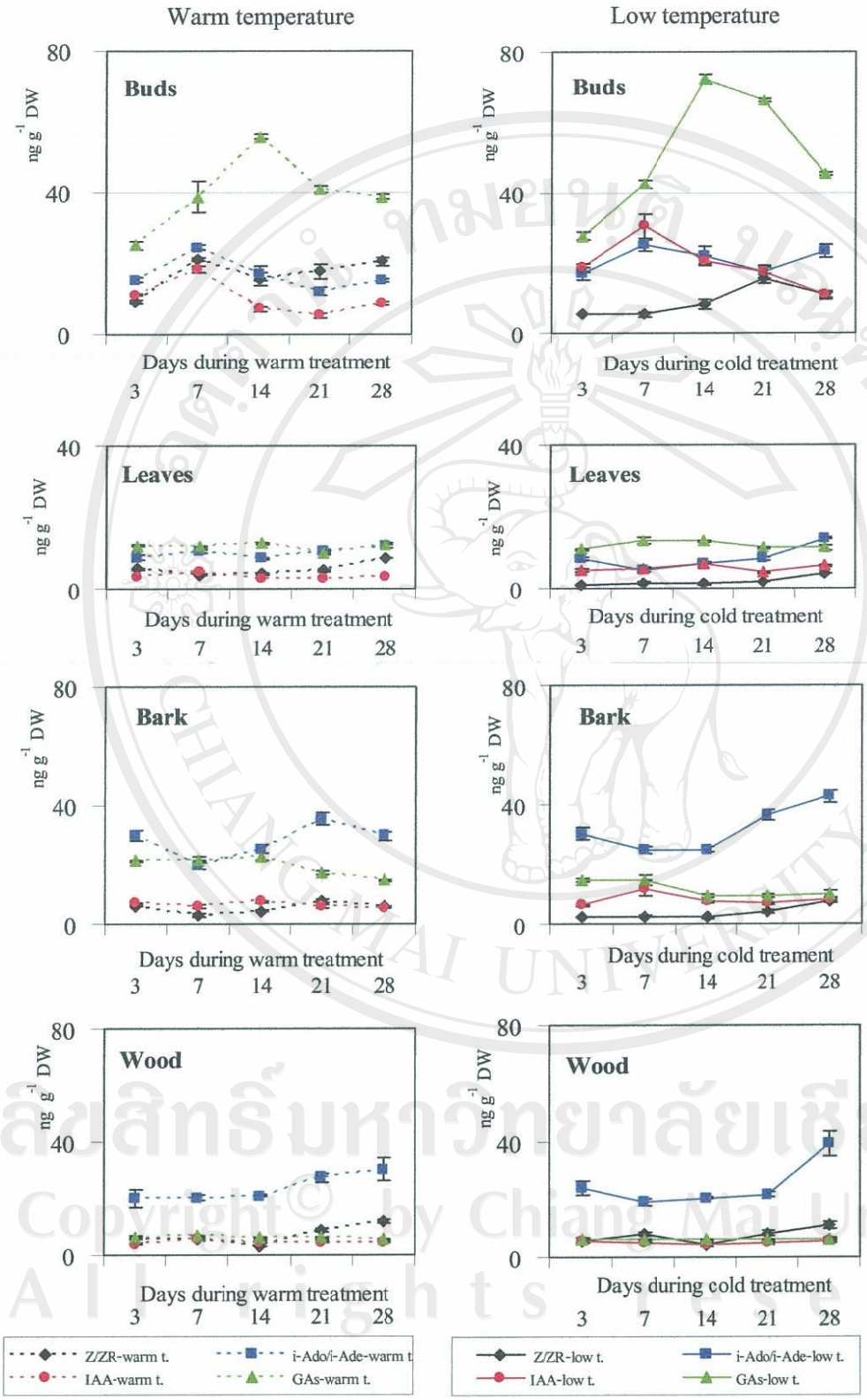


Figure 5.9 Hormonal concentrations in plant tissues at warm and low temperature conditions during the first month of treatments

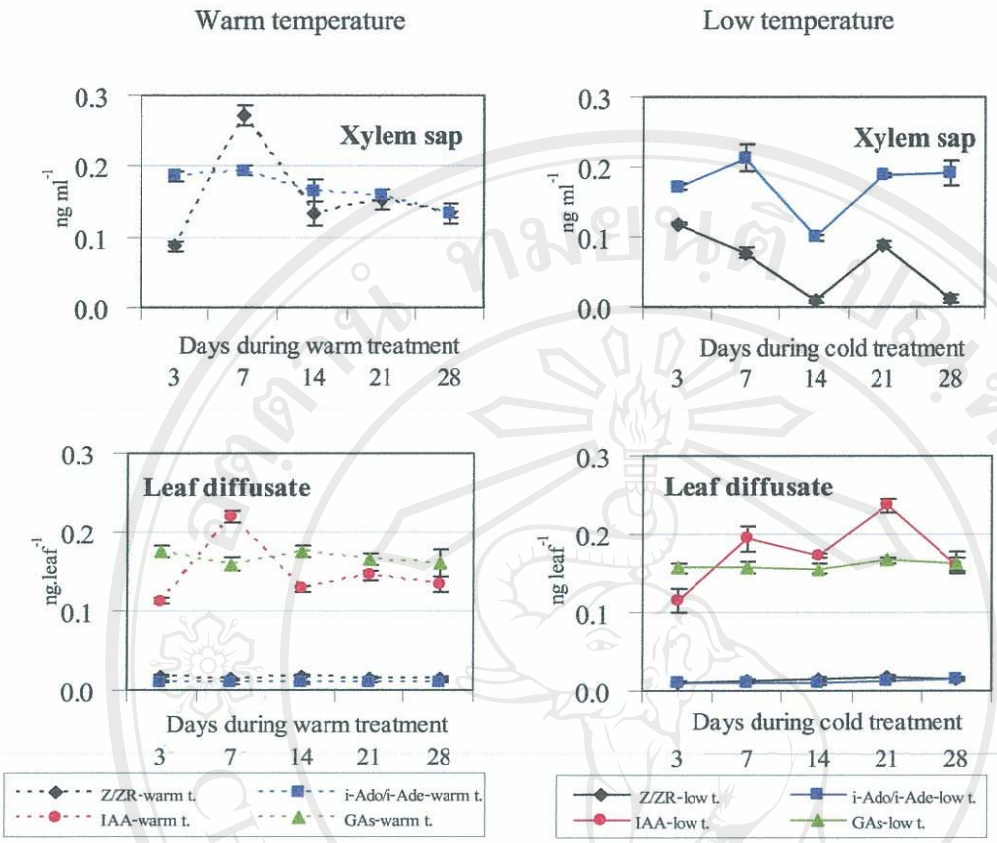


Figure 5.9 (Continued)

5.4 Discussion

The experiment in this chapter aimed firstly to confirm the results found in chapter 3 and secondly to understand deeper about the effect of low temperature on hormonal level in plant tissues and physiological activities, which concurrently the development of terminal buds. In chapter 3, it was found that:

1) A long, steady low temperature regime (15/10°C) of 38 days was more effective to promote flowering compared to 28 days cold duration.

2) Low temperature reduced diurnal change of photosynthetic rate, transpiration rate and stomatal conductance as well as chlorophyll fluorescence. In these parameters, plants showed response as fast as less than 7 days.

3) Low temperatures decrease Z/ZR level in terminal buds, bark, wood and xylem sap. A reduction in IAA level in leaf diffusate was also observed. Less effects of cold temperature on i-Ado/i-Ade and GAs were confirmed.

These findings should be discussed in this chapter.

5.4.1 Effect of low temperature on flowering of litchi tree

As found in chapter 3, low temperature had a strong effect on promote flowering in litchi tree, but only under two conditions of firstly enough cold (15/10°C) and enough long duration (38 days more effect than 28 days). This finding was confirmed in this experiment. Plants grown under 15/10°C temperature regime for 45 days produce visible active buds of 80% and within 18 days after rising up the temperature to be 27.5/17.5°C. Plants also started flowering within 24 days. In chapter 3, litchi plants started flowering within 37 and 27 days of warm weather after cold treatment for 28 and 38 days, respectively. Menzel (2002b) indicated that temperature affected the rate of reproductive development of litchi, with panicles emerging earlier at 15/10°C than at 20/15°C, but taking longer to reach anthesis Sukhvibul *et al.* (1999) stressed that the time taken for mango inflorescences to reach maximum length was 15-20 days on trees held at 25/15°C or 30/20°C whereas the trees kept at 20/10°C extended to 54 days. Moreover, Batten and McConchie (1995) also reported that the rate and duration of shoot growth was strongly related to temperature in mango and litchi grown in greenhouses.

We have to keep in mind that effect of growing condition affecting flowering in this experiment is not only the cold temperature but also the light intensity. In growth chamber, light intensity (*PAR*) was around 50% lower than that of greenhouse conditions. Anyway plants grown in growth chamber produced flower, whereas those grown in greenhouse produced flushing. This phenomenon confirmed a stronger effect of low temperature than light intensity on promoting flowering in litchi (Chen, 1990, 1991; Menzel and Simpson, 1994; Batten and McConchie, 1995; Naphrom *et al.*, 2001; Menzel, 2002a; O'Hare, 2004). Moreover, the same results also reported by Chen (1987), Whiley *et al.* (1989), Batten and McConchie (1995), Davenport and Nunez-Elisea (1997), Naphrom (2004) and Naphrom *et al.* (2004) on mango, Wong and Ketsa (1991) on longan.

5.4.2 Effect of low temperature on physiological activities and assimilate distribution

In chapter 3, it was shown that litchi trees response to low temperature very fast even less than 7 days of cold experience. *Pn*, *Tr*, and *Gs* decreased dramatically. However, less effect of

cold treatment on *Fv/Fm* was detected. All these findings were again confirmed in this experiment. But further studies on chlorophyll content in leaves, it was found that low temperature had no effect on chlorophyll content of leaves. This means that amount of chlorophyll content in leaves were not the limiting factor of photosynthetic efficiency of leaves.

The parallel decrease of photosynthetic rate, transpiration rate and stomatal conductance revealed that major factor limit the photosynthetic efficiency under cold temperature treatment was the opening of stomata. Under low temperature, stomatal opening was smaller than under greenhouse condition (Schaffer and Anderson, 1994; Nir *et al.*, 1997; Taiz and Zeiger, 1998; Allen *et al.*, 2000; Allen and Ort 2001). Such as in mango, dark chill-induced declined in midday photosynthesis was accompanied by increasing in stomatal limitation of light-saturated CO_2 assimilation and decrease in Rubisco activity. The stomatal closure may be caused by guard cell sensitivity to CO_2 following low temperature (Allen *et al.*, 2000; Allen and Ort 2001; Nir *et al.*, 1997).

Beside the low temperature effect on enzymatic activity as described in chapter 3, Browse and Xin (2001) emphasized a role of membrane fluidity as a primary signal upon a change in surrounding temperature. They speculated that a physical phase transition occurs in microdomains of the plasma membrane upon a downward shift in temperatures. Furthermore, Allen and Ort (2001) reported that reduced air and leaf temperature usually reduced evaporative demand. Cool roots also reduced hydraulic conductivity and substantially inhibit water uptake from the soil. However, respiration as the first approximation a nondestructive measure of metabolism responses significantly to change in temperature (Farrar *et al.*, 1996).

According to chlorophyll fluorescence measuring, normal ranges of *Fv/Fm* are between 0.75 and 0.85. A decline in *Fv/Fm* is a good indicator of photoinhibitory damage caused by light when plants are subjected to a wide range of environmental stresses including chilling, freezing and drought (Nir *et al.*, 1997; Haumann, 2002). Furthermore, Nir *et al.* (1997) reported that *Fv/Fm* declined in mid-day versus morning was 13%, while 43% in the chill-treated mango trees at 6°C from 0.79-0.81 at of untreated (23°C). Even though photochemical quenching and concentration of open PS II reaction centered response to light and CO_2 to meet a requirement for photosynthetic electron transportation (Chang *et al.*, 1999). However, temperature resulted in an imbalance between light energy absorbed through photochemistry *versus* the energy utilized

through metabolism. This energy imbalance was sensed through alterations in an excitation pressure of PS II, which reflected a relative reduction state of the PS (Hunner *et al.*, 1998). Furthermore, Savitch *et al.* (2001) reported that the photosynthetic capacity in *Arabidopsis thaliana* during cold stress as well as cold acclimation is altered by limitations at the level of consumption of reducing power (ATP) in carbon metabolism, which cold acclimation resulted in the stimulation of pentose phosphate pathway associated with limited capacity for carbon utilization through the starch biosynthesis. This overall resulted in the PS I acceptor side limitations associated with reduced $NADP^+$ availability (Aström *et al.*, 1998). For those reasons, *Fv/Fm* levels in this experiment as well as in chapter 3 may declined when trees exposed to low temperature compared to warm temperature. On the other hand, the photosynthetic apparatus of leaves may be impaired by cold temperature and repaired by rising temperature as indicated by *Fv/Fm* (Nir, *et al.*, 1997).

Stomatal movement is affected by potassium ion concentration in guard cells, water potential in stomatal cavity and epidermis cell, and also affected by plant hormone like ABA (Loveys and Kriedemann 1974; Taiz and Zeiger, 1998; Srivastava, 2002), as well as CO_2 concentrations in stomatal cavity (Nir, *et al.*, 1997; Taiz and Zeiger, 1998; Hicklenton *et al*, 2000; Srivastava, 2002).

In previous chapter, it was found a higher content of total non-structural carbohydrate, which was confirmed in this experiment as well, in leaves treated with low temperature. Accumulation of glucose or starch in leaves diminished the leaf water potential, which cause smaller stomatal opening. Similar to apple, Makhonpas (2001) studied on photosynthesis apparatus of leaves as influenced by water logging and fruit load. It was assumed that the lower photosynthesis and transpiration rates in non-fruiting than in fruit bearing trees is mainly due to the higher stomatal resistance as a result of high accumulation of carbohydrate in the leaves.

In this experiment, study on content of retardant hormone like ABA was not included. However, it should not be ruled out that under cold stress, litchi would also produce large amount of ABA, which lastly affected the stomatal movement. Cold stress and ABA accumulation have been reported elsewhere (Srivastava, 2002; Naphrom, 2004).

From the results showed in Figure 5.7, i.e. when rising up the chamber temperature, *Pn*, *Fv/Fm*, as well as *Tr*, *Gs* increased slowly. This suggested a direct effect of temperature on physiological activities of plants.

5.4.3 Effect of low temperature on hormonal change and relation to bud development

Most of the results in this chapter confirmed the finding of chapter 3, that low temperature decreased Z/ZR level in terminal buds, bark, wood and xylem sap. Less effects of low temperature on i-Ado/i-Ade and GAs were also confirmed.

The different results found would be IAA concentrations in buds and leaf diffusate, which increased under cold treatment. GA concentrations were also very high in buds and bark compared to the results from chapter 3. The results of this higher level of growth promoting hormone, e.g. IAA and GAs, even under low temperature treatment were based upon the different developmental stage of studied plants. Plants in this experiment were more mature, which produced flushing within 14 days of the study period. Plants in chapter 3 produced flushing within 29 days.

In this experiment, terminal bud development remained vegetative bud even at 3-4 weeks low temperature treatment. This is closely link and related to the theory, that in cold treated terminal bud a relative high GA concentrations in buds were found and at the same time IAA concentrations increased in buds, leaves and leaf diffusate, this result supported Ross *et al.* (2000) and Ross and O'Neill (2001), which reported that normal auxin concentrations, IAA were required to maintain normal levels of GA_1 in elongating pea stems, so that interaction of IAA and GAs may involve in litchi tree. Moreover, Bangerth *et al.* (2000) also suggested that basipolar auxin may control CK production in the roots and it possible delivery to lateral buds.

According to reproductive bud development required a low level of GAs (Tongumpai *et al.*, 1991; Goldschmidt *et al.*, 1997; Koshita *et al.*, 1999; Qiu *et al.*, 2001; Hegele *et al.*, 2004; Naphrom *et al.*, 2004) and auxin (Bernier *et al.*, 1993; Hegele *et al.*, 2004) but high level of cytokinins (O'Hare, 1989, 2004; Chen, 1990, 1991; Stern *et al.*, 2003; Hegele *et al.*, 2004; Naphrom, 2004; Naphrom *et al.*, 2004). For genetic of flowering, however, several physiologists reported that several genes required to mediate transition to flowering were detected in *Arabidopsis* and other species (Levy and Dean, 1998; Colasanti and Sundaresan, 2000; Bernier

and Périlleux, 2005). Some genes act in leaves to produce a floral stimulus or inhibit a promoter of vegetative development (Colasanti and Sundaresan, 2000). Other genes act at shoot apex to mediate responsiveness to external signals. However, the genetic networks that interact to regulate flowering are being elucidated by analyzing flowering-time genes from *Arabidopsis*, maize and other species. Bernier and Périlleux (2005) reported about flower induction that all plants parts participate in the sensing of these interacting factors. In *Sinapis alba*, it involve the root system and to include sucrose, nitrate, glutamine and cytokinins, but not gibberellins. Moreover, it was proposed that a model controlling flowering time in early-flowering accessions of *Arabidopsis* involves metabolites, hormones and gene products interacting as long-or short-distance signaling molecules. A new perspective on this flowering is to consider the plant as a multi-component system comprising the shoot apical meristem (the target of the signal), the leaf (the floral-signal source) and the plasmodesmata and vascular tissue (the signal movement) (Bernier *et al.*, 1993; Colasanti and Sundaresan, 2000; Bernier and Périlleux, 2005).

5.5 Conclusion

1) Low temperature had a very strong effect to promote flowering in litchi tree, but with three important cofactors e.g. enough cold of 15/10°C, enough duration of 38-45 days and developmental stage of shoot (not fully mature) at the time expose to low temperature.

2) Low temperature increased assimilates accumulation in leaves and decrease stomatal aperture which then diminished photosynthetic rate and transpiration rate. No effect of low temperature on leaf chlorophyll content was found.

3) Low temperature decreases Z/ZR in buds, leaves, bark, wood and xylem sap, whereas less effects of low temperature were on i-Ado/i-Ade. GAs increase slightly in buds, but it was not obvious affected in other plant tissues. IAA level increased slightly in buds, leaves, leaf diffusate and bark. Effect was not clear on i-Ade/i-Ade level, which it should be further confirmed.