Chapter 7

General Discussion and Conclusion

7.1 General discussion

7.1.1 Appropriate duration of low temperature to promote inflorescence bud development

Low temperature had a very strong effect to promote floral bud development in litchi tree, but with three important cofactors, e.g. adequate low temperature of 15/10°C, adequate cold duration of 38 days, and lastly the developmental stage of leaves (not fully mature) at the time exposed to low temperature. However, active inflorescence buds also occurred in low temperature regime (15/10°C) of 28 days even though it was less effective to promote flowering than 38 days of cold duration. It can be concluded that litchi plant cv. 'Hong Huay' required 38 days cold treatment, for which the 100% active inflorescence bud could be achieved under cold temperature of 15/10°C treatment. The results supported Menzel's suggestion (2002b) that the best condition for flowering was at least three weeks of low temperature. And it also supported the finding of Batten and McConchie (1995) and O' Hare (2004) that litchi need 39 days of transfer to low temperature. In addition to above mentioned cofactors for success flowering, the terminal buds seem to require mild warmer temperature of 27-29/23-24°C for another 11-18 days to promote successful inflorescence meristems/buds development.

In this study, plants were kept under constant temperature in growth chambers. The low temperature was operated at constant temperature 15/10°C. Under natural condition, this steady low temperature condition is very hard to achieve. That is the major problem causing irregular or even alternate bearing in litchi. Improvement the sensitivity of litchi tree to low temperature to shorten the chilling requirement for bud stratification or floral bud induction may be the field practice to be developed. Details on this matter will be discussed later. But the key success will be based upon the best understanding of the physiological response of plant to low temperature, in especially in relation to flowering.

7.1.2 Mechanism of physiological responses and some biochemical changes of litchi when flowering under low temperature

7.1.2.1 Photosynthesis, chlorophyll and assimilate distribution

Low temperature reduced diurnal photosynthetic rate and chlorophyll fluorescence. Litchi leaves response as fast as less than 7 days of exposure to low temperature even though no effect of low temperature on leaf chlorophyll content was found. However, the photosynthetic rate and chlorophyll fluorescence increased slightly when the temperature was rising up, so that it may indicate that the photosynthetic apparatus of leaves may be impaired by low temperature and repaired by rising temperature as mentioned by Nir *et al.* (1997) and Lawlor (1993). Although an effect on photosynthesis was found, but cold temperature increased assimilate accumulation in leaves even not in roots. This indicates a low assimilate distribution due to low temperature, especially a long distance transport to root. This negative effect of low temperature on night mobilization of starch from leaves was reported by Paul *et al.* (1992).

A closer study on the effect of low temperature on assimilate accumulation in leaves and roots was made based on "percent difference low temperature /warm temperature" calculation, which it was calculated by percentage of difference (low temperature/warm temperature) = 100 – [(100*warm temperature) / low temperature] as shown in Figure 7.1, TNC in leaves increased steady along with the cold temperature duration, whereas RS level remained similar to warm temperature. By rising up the temperature both TNC and RS in leaves reduced steady, as a result of more mobilization of carbohydrate out of leaves to support floral bud development (differentiation) at day 4-18 of rising up the temperature. During these period photosynthetic rate increased at a very low extent (Figure 6.6).

Increase in ambient temperature also decreased the TNC and RS level on day 11-18 compared to he level under warm temperature (Figure 6.7). Similar to mango, Nartvaranan (1997) reported that gradually decreasing TNC and RS content in leaves and bark during visible floral bud to full bloom. Normally, carbohydrate reserves are often mentioned as one of prerequisites for floral induction in tropical in litchi (Koo-Duang, 1984; Menzel *et al.*, 1995; Thonglem, 2000), longan (Pichakum *et al.*, 2003; Kiatsakun, 2004) and other species (Goldschmidt, 1999; Ulger *et al.*, 2004). Such in litchi, Thonglem (2000) reported that TNC in stem apex increased in the 4th week prior to flowering. And Menzel *et al.* (1995) also indicated

that starch contents were about double those in leaves, twigs, small branches and medium branches of flowering tree with floral buds just visible when compared with litchi trees in early-mid flush. Furthermore, in 'Daw' longan treated with $KClO_3$, TNC concentrations in root declined while it increased in terminal shoots during floral initiation through floral development (Kiatsakun, 2004). At this study period a warm temperature also activated the root metabolism especially to produce necessary substances for floral development, e.g. cytokinins (Z/ZR), which large amounts were detected in xylem sap. Such a metabolism required carbohydrate as an energy source. TNC and RS were the most effective raw material. According to the interaction between the increase in carbohydrate and the increase in CK has been observes at the level of the apical meristem (Bernier, *et al.*, 1993; Goldschmidt, 1999). Furthermore, studies on the changes in the levels and transport of endogenous hormones and saccharides during flower induction showed their possible involvement in the regulation of flowering (Macháckova *et al.*, 1996; Havelange *et al.*, 2000).

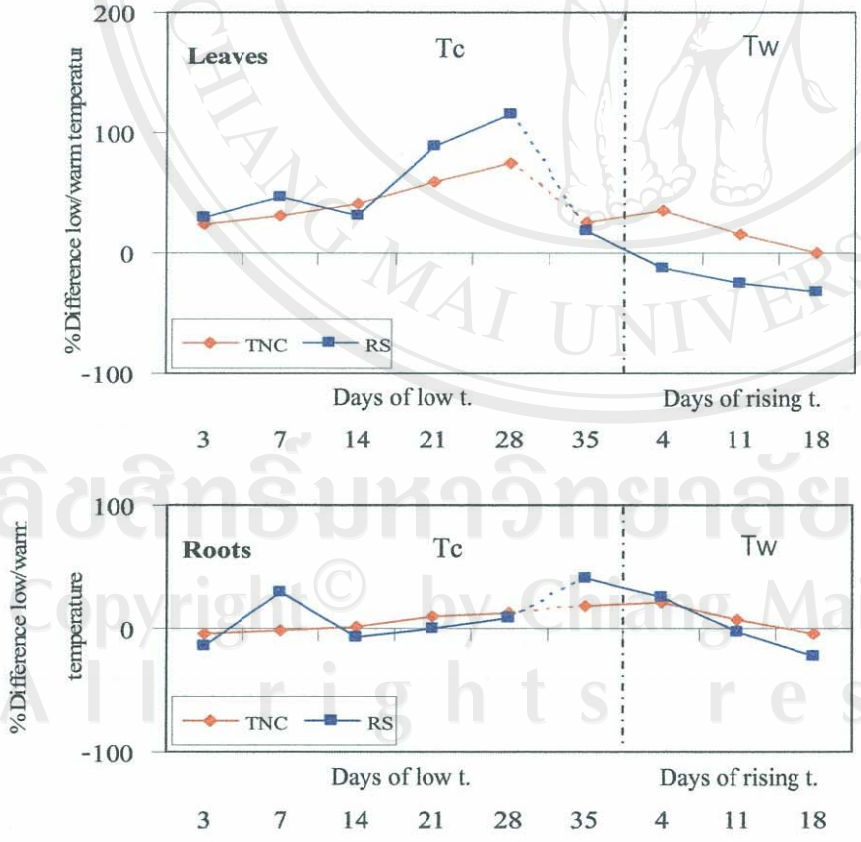

Figure 7.1 Difference of TNC and RS concentrations in leaves and roots between low and warm temperature

7.1.2.2 Transpiration and stomatal conductance

When litchi trees exposed to low temperature, the reductions of leaf temperature, stomatal conductance and transpiration rate were found in leaves. Their values however increase slightly when the temperature was rising up.

In this case, the smaller opening of stomata was the major factor limiting transpiration as well as the photosynthetic efficiency under low temperature treatment. Under low temperature, stomatal opening was smaller than under warm temperature condition (Nir *et al.*, 1997; Taiz and Zeiger, 1998; Allen *et al.*, 2000, Allen and Ort 2001). The stomatal closure may be caused by a sensitivity of guard cell to CO_2 caused by low temperature. Furthermore, a stress condition of low temperature may activate the litchi tree to produce and accumulate large amount of ABA as in mango (Srivastava, 2002; Naphrom, 2004), so that it affected the stomatal movement. Moreover, low temperature was also reported to increase assimilate accumulation in leaves (Farrarrr and Gunn, 1996; Strand *et al.*, 1997), so that it may effect a reduction in water potential and lastly decrease stomatal aperture, which then diminished photosynthetic rate and transpiration rate (Brüggemann, *et al.*, 1992; Farrarrr and Gunn, 1996; Sun *et al.*, 1999).

7.1.2.3 Physiological response in the aspect of hormone balance

It was clearly concluded from the research result that low temperature promote flowering in litchi *via* its effect on reduction of IAA but on increase of Z/ZR in terminal bud. The percentage difference low/warm temperature was again calculated for a better follow up the effect of low temperature on hormone balance in different plant tissues.

1) Hormone balance in plant tissues

As shown in Figure 7.2 that low temperature treatment may firstly increase some growth regulators, e.g. IAA, GAs, i-Ado/i-Ade compare to tissues under warm temperature. However, at the late duration of cold treatment (day 28-38 of cold treatment), these growth regulator level dropped down. In contrast, the level of cytokinin (Z/ZR) increased dramatically after cold treatment and during temperature rising period, which it synchronized with the floral differentiation period. Increase of Z/ZR level in bud at day 4-18 of warm temperature may be due

to a significant supply from root through xylem sap (Figure 7.4), from bark and wood (Figure 7.3).

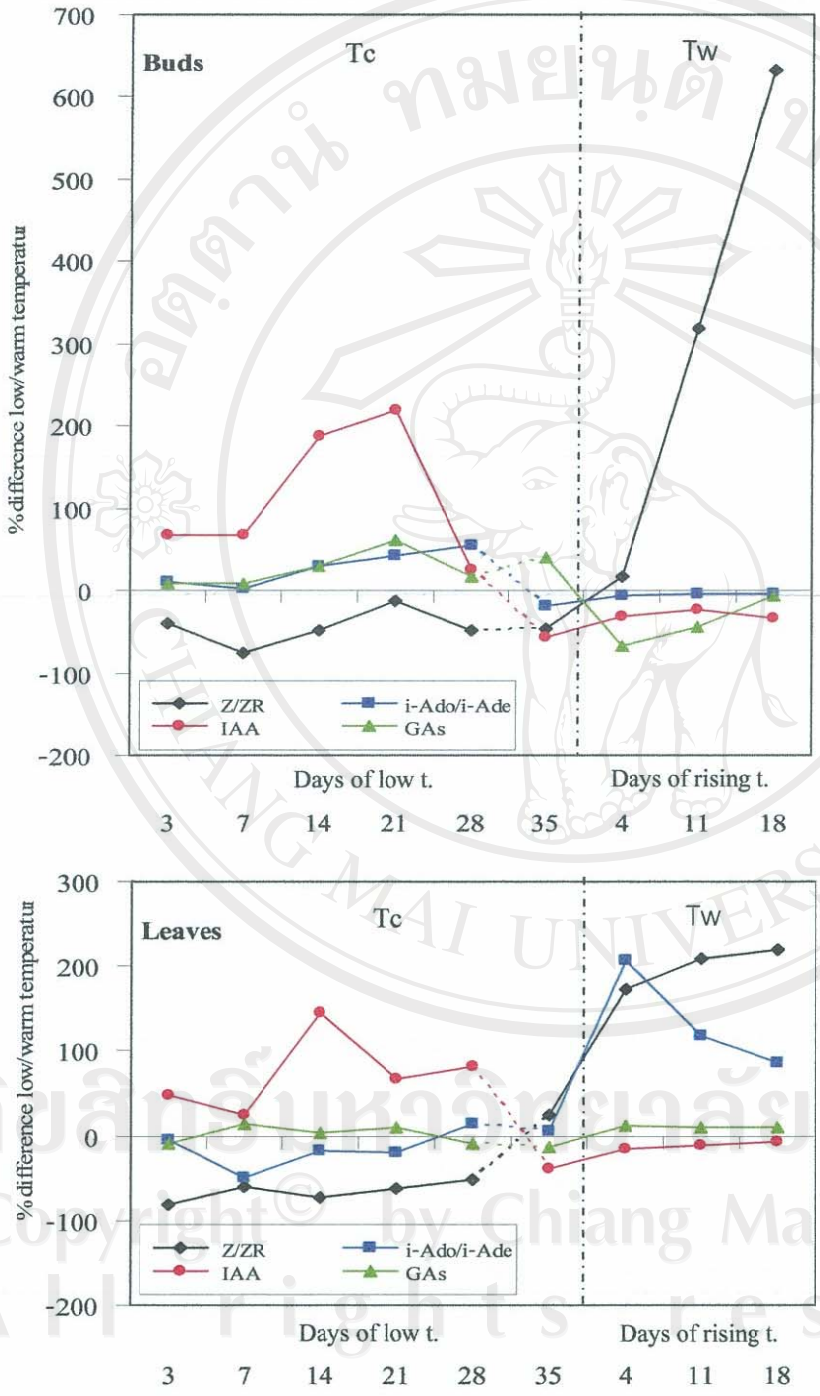

Figure 7.2 Difference of hormonal concentrations in buds and leaves between low and warm temperature

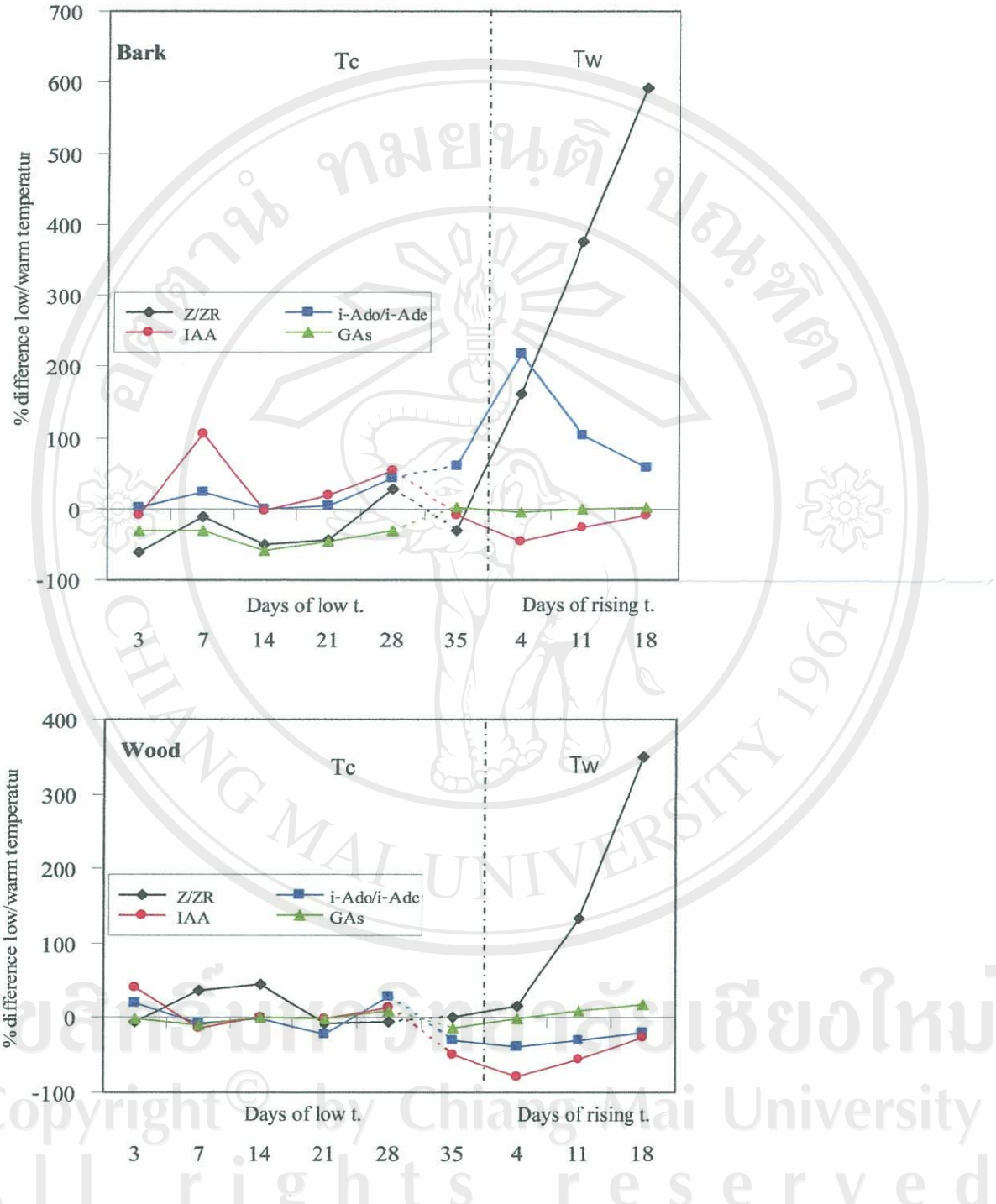

Figure 7.3 Difference of hormonal concentrations in bark and wood between low and warm temperature

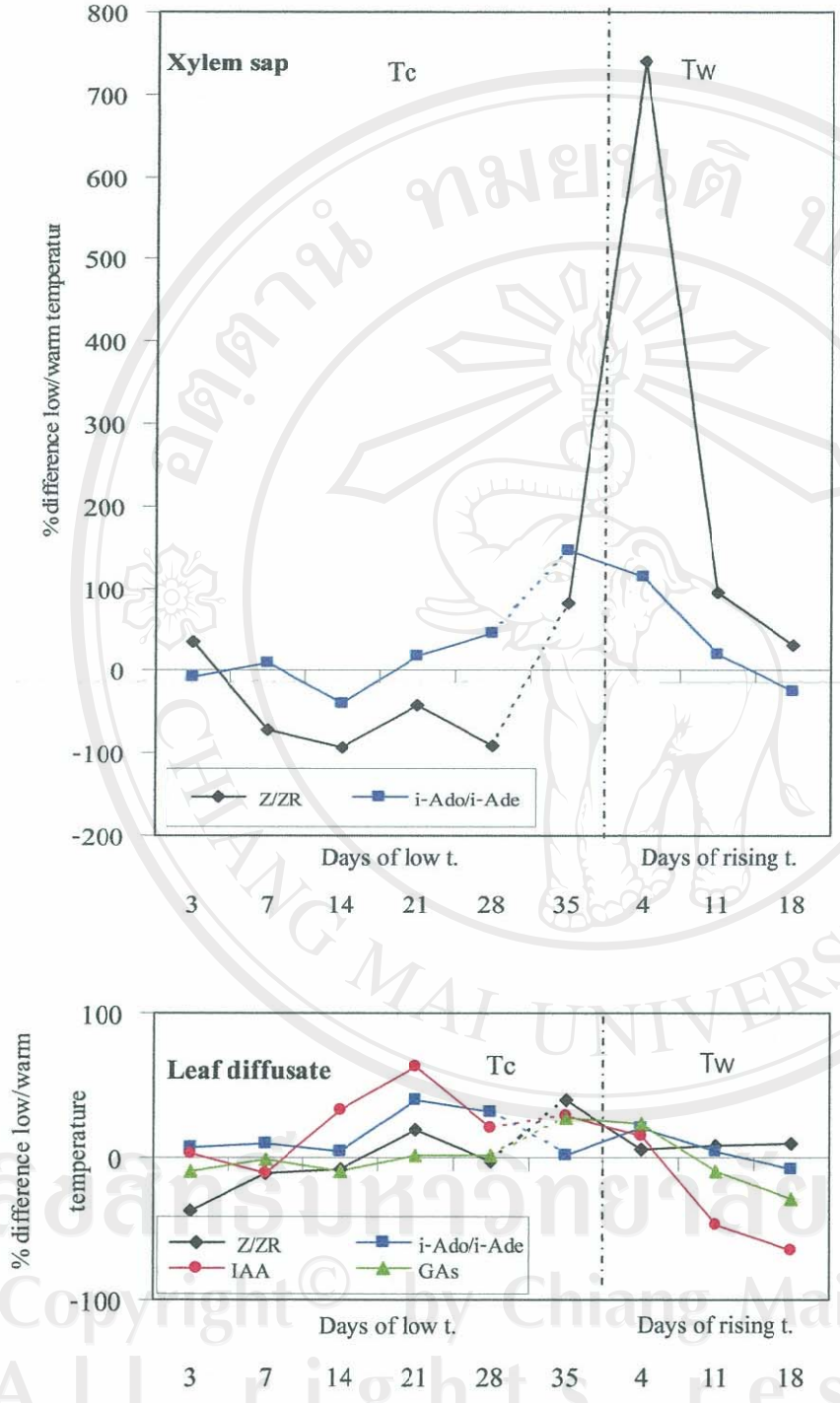

Figure 7.4 Difference of hormonal concentrations in xylem sap and leaf diffusate between low and warm temperature

2) Model of hormonal transport in plant under warm or low temperature

From the results mentioned above, comparing between low temperature and warm temperature condition, the results can be developed separately into model of hormone transport among plant tissue in three schemes of the developmental stage of litchi tree.

2.1) Model of hormone transport under warm conditions

Litchi trees grown under warm temperature condition usually stayed in vegetative growth period, it might be concluded that intermediate CKs concentrations translocated from the roots *via* xylem sap and leaves *via* phloem to the terminal buds (Figure 7.5 A). Similarly, Dewitte *et al.* (1999) studied on distribution in tobacco (*Nicotina tabacum* L.) shoot apices in distinct phases of development using immunocytochemistry and quantitative tandem mass spectrometry. They concluded that during organ formation (e.g. leaves and flowers), was characterized by enhanced free CK content. In contrast in longan, Chen *et al.* (1997) reported that in the transition of the terminal bud from leaf flush to dormancy, the principal CKs were conjugate forms, i.e. zeatin-O-glucoside and zeatin riboside-O-glucoside. CKs which translocated to the shoots were accumulated in the buds at dormant stage. In addition, i-Ado as a precursor of Z/ZR is also believed to convert to trans-Z *via* i-Ade or trans-ZR (McGaw and Burch, 1995). ZR, predominant CKs, transports from roots *via* xylem, while Z is a major transported form in phloem of *Ricinus* that determined by GC-MS (Baker, 2000).

In these experiments high auxin (IAA) concentrations in the buds was translocated basipetally to the lower parts of the tree, whereas low IAA concentrations in leaves may translocate slightly *via* phloem to the junction of petiole and stem which it might occur low axin transport autoinhibition (ATA) (Figure 7.5 A). It supported a suggestion of Bangerth *et al.* (2000) that differences in IAA export from, and transport capacities of, dominant and dominated shoots, may be explained by a mechanism of ATA, whereby the earlier and stronger export of IAA from the dominant shoot inhibits auxin exported from the dominated shoot at the point where the two auxin streams converge.

Furthermore, high GAs concentrations translocate through phloem and xylem to the litchi buds and leaves (Figure 7.5 A). Recently, Ross *et al.* (2000) suggested that normal levels of the

auxin, IAA were required to maintain normal levels of bioactive GA (GA_1) in elongating pea stems, so that interaction of IAA and GAs may also concern in litchi tree.

2.2) Model of hormone transport under low temperature treatment

Litchi trees subjected to low temperature (15/10°C), it is divided into two periods. Firstly, during the five weeks of low temperature as floral induction stage, it may conclude that low CK concentrations translocate *via* xylem sap and phloem through the terminal buds and the leaves and accompanied by dormant terminal buds (Figure 7.5 B). In contrast, the effects of low temperature (13°C) on 'Tommy Atkins' mango were indicated that Z/ZR concentrations in terminal buds, bark and wood were greater than at warm temperature (25°C) during days 13-29 of treatment (Naphrom, 2004; Naphrom *et al.*, 2004). Furthermore, mango trees at cool winter, CK-like also increased in xylem sap during early floral induction (Chen, 1987). However, these results were similar to 'Hong Huay' litchi trees grown in Doi Pui orchard, Chiang Mai. Naphrom *et al.* (2001) reported that low CK-like concentrations were found during 6-8 weeks prior to flowering. Similarly, Chen (1991) investigated that CK concentrations in buds of 'Heh yeh' litchi were low during bud dormancy prior to floral initiation and the buds did not respond to exogenous CK application.

In these experiments, IAA level increased in buds, leaves and leaf diffusate (Figure 7.5 B). Similar to mango during days 13-29 of cold treatment, great IAA level were found in leaf diffusate, whereas IAA in terminal buds had no obvious effect (Naphrom, 2004; Naphrom *et al.*, 2004). However, a constant level of IAA in shoot tips of 'Heh yeh' litchi was maintained through the stages of leaf expansion, bud dormancy until full bloom (Chen, 1990). In addition, low temperature might inhibit IAA polar transport out of the shoot tip in pea (Morris, 1979), mango (Naphrom *et al.*, 2004) and the subtropical trees (Davenport and Nunez-Elisea, 1997), even though it was not investigated in these experiments of litchi.

Moreover, intermediate auxin concentrations in the terminal buds and high concentrations in the leaves may occur and it had high ATA at the junction between petiole and stem. In addition, Bangerth *et al.* (2000) revealed in explants of pea, apple and tomato that the basal application of cold IAA significantly reduced endogenous as well as exogenous IAA transport through these explants. In addition, interaction between low CKs and high auxin

concentration seems to occur, which Bangerth *et al.* (2000) indicated that the interruption of the polar auxin transport leads to a strong increase CKs in root.

Furthermore, in chapter 4, it was revealed that high auxin is synthesized in the young leaf and exported to the other parts of the tree. Whenever a new shoot with leaf flush occurs short before the flowering period, high concentrations of auxin in the young leaves may inhibit flower bud formation. Bangerth (1997) suggested that strong vegetative growth inhibited floral induction while methods reducing growth, i.e. bending of shoots, stimulated and GAs would exert their inhibiting function in floral induction possibly *via* a stimulation of IAA biosynthesis at a site of signal generation. In these experiments, intermediate GAs concentrations were found through the buds and leaves *via* phloem and xylem (Figure 7.5 B), which it may synchronize with IAA concentrations as described above.

In generally, however, litchi trees require lower temperature and longer cool period than mango and longan trees for floral induction. Whenever temperature in winter is not cool enough for litchi to enhance flowering, but mango and longan trees can flower. Similarly, Whiley *et al.* (1989) reported that temperature below 20°C enhanced floral induction in mango, whereas longan trees require 15-22°C in winter season for 2-3 months (Wong and Ketsa, 1991).

2.3) Model of hormone transport during temperature rising period

During 4-18 days of temperature rising period an inflorescence bud seems to emerge as floral differentiation Z/ZR and intermediate i-Ado/i-Ade concentrations were found in all parts of plant tissues, whereas low IAA concentrations in the buds and intermediate concentrations in the leaves accompanied by low ATA. Low GA concentrations were also found in the terminal bud and leaves (Figure 7.5 C).

It is also known that CKs promoted inflorescence and flower bud differentiation in fruit species, for example, litchi (Chen, 1990, 1991; Naphrom *et al.*, 2001; Stern 2003), longan (Chen *et al.*, 1997; Hegele *et al.*, 2004), Japanese pear (Ito *et al.*, 2001) and mango (Naphrom, 2004; Naphrom *et al.*, 2004). Such as in 'Hong Huay' litchi trees, Naphrom *et al.* (2001) found that The CK-like concentrations increased at four weeks to reach a maximum two weeks prior to flowering as well as in longan, Chen *et al.* (1997) found a large decrease in CK glucosides and an increase in Z and ZR activities in bud during floral initiation.

Moreover, interaction between high CKs and low auxin concentrations occur. It supported by Hoad (1984) which CKs seemed to act as IAA-antagonists in flower bud initiation as well. Applied in the vicinity of potential flower buds they stimulate floral induction Similarly, Bangerth (1994) also mentioned that reduced polar IAA transport into the root would be another possibility for high xylem CK.

During this period, low GA levels were also found in the terminal bud and leaves of litchi (Figure 7.5 C). It was similar to the findings which several researchers reported that low temperature induced flowering accompanied by a decrease in endogenous GA concentrations in mango (Tongumpai *et al.*, 1991; Naphrom *et al.*, 2004), longan (Qiu *et al.*, 2001) and citrus (Goldschmidt *et al.*, 1997; Koshita *et al.*, 1999). Therefore, it may conculde that GAs inhibited inflorescence bud formation in this period.

Therefore, these results may also conclude that before flowering through the stage of inflorescence bud appearance the terminal bud produces a lower concentration of auxin, whereas the xylem sap, the leaves and the terminal bud produce a high concentration of cytokinin.

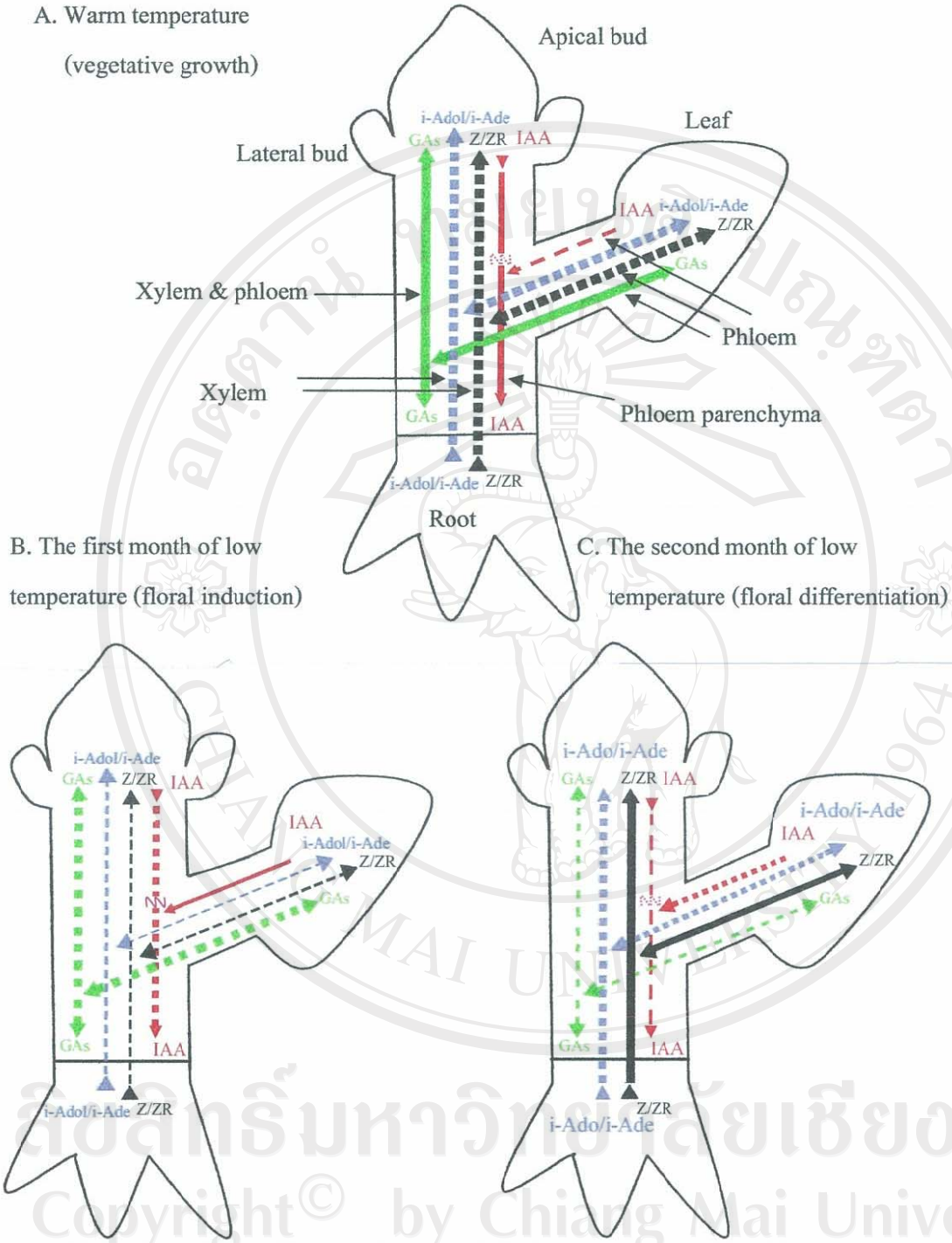

Figure 7.5 Scheme of hormonal changes in litchi trees under warm and low temperature conditions (Solid arrows indicate high concentrations. Bold dotted and thin dotted arrows indicate intermediate and low concentrations, respectively. Solid and dotted wavy lines indicate high and low auxin transport autoinhibition (ATA), respectively.) (modified from Naphrom, 2004)

7.1.3 Relationship between terminal bud development and changes in physiology and biochemistry

As already discussed elsewhere, low temperature limited activity of photosynthesis and transpiration, stomatal opening, leaf temperature, which similar to the case of mango and subtropical woody species (Schaffer and Anderson, 1994; Nir *et al.*, 1997; Allen *et al.*, 2000; Hendrickson *et al.* 2004). Low temperature as a stress condition fro these trend enhanced ABA concentration, which then causing stomatal closure. Photosynthetic rate decreased concurrently due to its dependence on stomatal opening and intercellular CO_2 concentration in plant as well as enzymatic activities (Kingston-Smith *et al.*, 1997; Srivastava, 2002; Stitt and Hurry, 2002).

Furthermore, low temperature also resulted in reduction of electron transport ability (Brüggemann and Dauborn, 1993; Nir *et al.*, 1997; Astrõm *et al.*, 1998; Cavaender-Bares *et al.*, 1998) which lastly reduced of CO_2 assimilation rates. Low temperature may also influence membrane fluidity (Browse and Xin, 2001) and inhibit mobilization rate of sucrose out of leaves (Paul *et al.*, 1992; Brüggemann *et al.*, 1992; Sun *et al.*, 1999), so that TNC in litchi leaves increased. Moreover, reduction on enzymatic activities was affected by low temperature. A low respiration rate increased assimilates accumulate in plant tissue such as leaves (Lawlor, 1993). Thereafter feedback effect of high carbohydrate accumulating in leaves limits photosynthesis process (Farrar and Gunn, 1996; Strand *et al.*, 1997; Iglesias *et al.*, 2002).

For terminal bud development, terminal buds of litchi seemed to remain dormant during the first month of cold period then became active and developed to be inflorescence buds by rising up the temperature. Carbohydrate (sucrose) accumulation in plant tissue especially in leaves may act as carbon source supply to the meristem tissue to activate the energy-consuming process such as mitotic activities as reported in *Sinpis alba* (Chacko, 1991; Bernier *et al.*, 1993; Lejeune *et al.*, 1993; Davenport and Nunez-Elisea, 1997; Levy and Dean, 1998; Corbesier *et al.*, 1998). Ohto *et al.* (2001) even reported that sugar may affect floral transition by activating or inhibiting genes that control floral transition.

Sugar is an important raw material for CK, GA and ABA biosynthesis which cascade process are derived from mevalonic pathway (Davies, 1995), so that sugar also strongly regulate the plant development. Furthermore, active growth of tissues relied on the function of the cell division cycle. A central control point of the cell cycle is the transition between the G1- and

S phase, and extracellular signals are linked to this transition via D-type cyclins, which regulated both by sugars and CKs (Murray *et al.*, 1998 cited in Roitsch and Ehneß, 2000). In addition, induction of extracellular invertase, ionically bound to cell wall irreversibly hydrolyses the transport sugar sucrose, may integrate the regulation by CKs with other stimuli resulting in a sugar signal that mediates further downstream responses and could provide a link between cytokinin responses and metabolism (Roitsch and Ehneß, 2000).

Relation between terminal bud development and physiology and biochemical change could be divided according to the bud section studies into three critical stages (Figure 7.6). Firstly, at dormant bud stage (during 7-28 days of cold treatment) the bud visibly non active, by which TNC and RS accumulation in leaves were higher than control plants, which supported the previous works of Pichakum *et al.* (2003) and Kiatsakun (2004) in longan and Koo-Duang (1984), Menzel *et al.* (1995), Hieke (2000) and Thonglem (2000) in litchi. For plant hormone content in buds, leaves and roots; Z/ZR were lower in all plant tissue compared to control plants; IAA and GAs remained, however, higher both in leaves and buds. High auxin concentrations may interrupt CKs transport from root (Bangerth, 1994, 1997; Bangerth *et al.*, 2000). Ross *et al.* (2000) and Ross and O'Neill (2001) reported the high GA concentrations in buds induced by high concentrations of IAA.

Secondly, at quiescent bud stage (day 35 of cold treatment until day 4 of temperature rising up), terminal bud remained dormant, but with active development at tissue level. TNC and RS in leaves decreased with the possibility of retranslocation of assimilate and sucrose for energy source and for metabolic pathways from leaves to active buds. At this stage IAA and GAs decreased but more Z/ZR translocate from root through xylem sap was detected. Under 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. This period a low level of GAs seemed to be neccessary (Tongumpai *et al.*, 1991; Goldschmidt *et al.*, 1997; Koshita *et al.*, 1999; Qiu *et al.*, 2001; Hegele *et al.*, 2004; Naphrom *et al.*, 2004) as well as auxin (Bernier *et al.*, 1993; Hegele *et al.*, 2004).

Thirdly, at bud swelling and protrusion stage (day 11-18 of temperature rising up) a clear visible active bud stage was observed. Internally, a complete development of inflorescence tissue was observed and externally inflorescence bud started to open. At this stage large amount

of carbohydrate was required, so that lower content of TNC and RS in leaves were measured compared to control plants. This result supported the work of Stephenson *et al.* (1989) that carbohydrates in bark of macadamia trunk declined with the onset of the spring flush and flowering. Significant increase in Z/ZR was found in terminal bus, leaves, wood, bark and root (xylem sap), whereas auxin and GAs remained at as low concentration as control plants. In addition, 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). In addition, low concentrations of auxin in bud were also support Bernier *et al.* (1993) and Hegele *et al.* (2004).

Furthermore, plant development, physiology and metabolism are regulated by input from a number of signaling/response pathways, which include those involved in response to phytohormones, environmental stimuli and metabolites such as sugars and nitrogen as forming an interconnected web (Gibson, 2000; Bernier and Périlleux, 2005). This signal that affects one part of the web can then affect other parts of the web, more or less strongly and directly (Gibson, 2000).

In conclusion, these findings confirmed the close relation between carbohydrate balance, hormonal balance and bud development. In general, low temperature promoted flowering in litchi accumulation of TNC and RS in leaves and bud, and secondly the shifting of hormonal balance. In the last condition, Z/ZR was firstly decreased with increased of IAA and GAs to induce reproductive bud formation and development. Shortly before bud swelling a bud burst high concentrations of Z/ZR was then accumulated in bud through Z/ZR translocation from root and leaves, whereas IAA and GAs decreased. These phenomena required an adequate warm temperature, which should be happened immediately after cold period of 15/10°C at around 28-35 days.

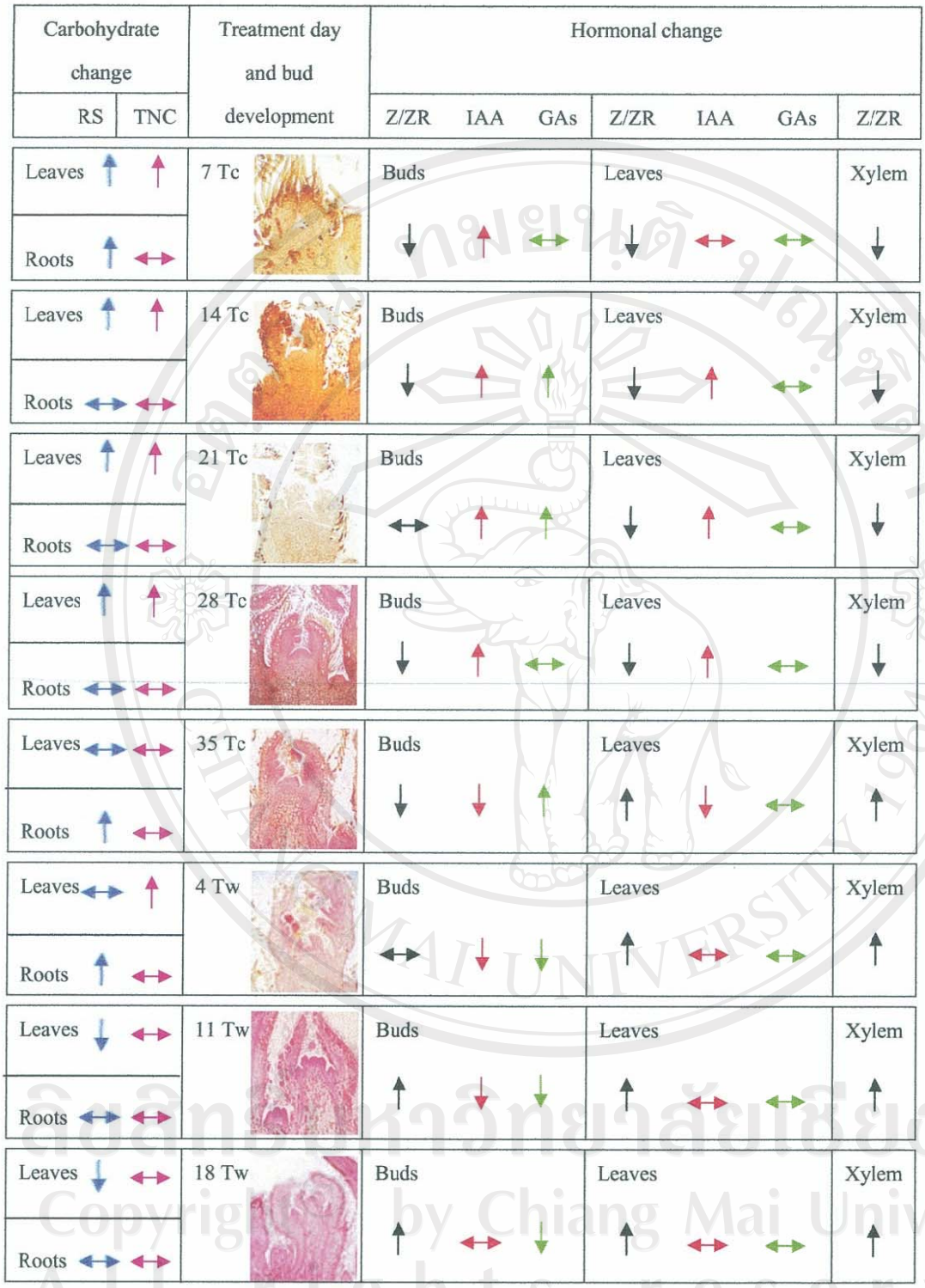

Figure 7.6 Overview of relationship between terminal bud development, physiological and biochemical changes of litchi tree when expose to low temperature treatment

7.2 Conclusion

This study on effect of low temperature treatment on flowering, physiological and biochemical change in litchi showed that:

1) Low temperature had a very strong effect to promote flowering in litchi tree. 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 at tissue level by flattening of apical meristem and enlargement of leaf primordial, which already took place within 4 days of warm 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 axillary bud. Only at this stage the bud swelling would be firstly observed.

3) Low temperature increased assimilates accumulation in leaves and decrease stomatal aperture which then diminished chlorophyll fluorescence, photosynthetic rate and transpiration rate. Plants showed response as fast as less than 7 days. No effect of low temperature on leaf chlorophyll content as well as assimilates concentrations in roots was found.

4) During the first month of low temperature treatment Z/ZR content decrease in buds, leaves, bark, wood and xylem sap. Less effects of low temperature were measured on i-Ado/i-Ade. IAA level increased slightly in buds, leaves, leaf diffusate and bark, whereas GAs increased slightly in buds.

5) After expose to low temperature, a rise up of temperature will increase CKs in all plant tissues, whereas IAA and GAs decrease. This occurred at the time of floral differentiation period.

7.3 Suggestions for future experiment

These experiments were investigated on only active form of hormones, such as Z, ZR, i-Ado, i-Ade, IAA, GA_1, GA_3 and GA_{20}. During experimental period, the pattern of hormonal changes did not move smoothly in only one direction, but it fluctuated during plant development period. Conjugated form of those hormones and their balance may relate closely to hormonal effect on flowering. This should be further studied. Moreover, effect of hormone on floral "induction" did not confirm in this study, it may study on change in cytomorphology, DNA and

tRNA at the beginning of transition from vegetative bud to reproductive bud. Changes of TNC, RS, ABA, ethylene and nutrient such as Ca^{2+} as well as polar auxin transport from the shoot tips also should be studied under low temperature compared with warm temperature condition. These informations should be very beneficial for a better understanding on effect of low temperature on flowering of litchi.

7.4 Suggestions for field application

As mentioned in conclusion that during preseason of flowering of litchi, low temperatures increase carbohydrate (TNC), Z/ZR and decrease IAA and GAs content in plant tissues. In addition, IAA concentrations exported from young leaves were greater than old leaves. Therefore, knowledge transfer for field application may be suggested as following:

7.4.1 Shortly prior to flowering, the young leaves or new leaves not be allowed, it may be manipulated by girdling, chemical application or integrating both techniques. However, if new shoot occurs in this period, high endogenous auxin concentration produced in young leaves may inhibit floral bud formation, so that trimming of new flush should be practiced.

7.4.2 Effect of low temperature on floral induction could not be replaced right now. A high concentration of carbohydrate accumulation in plant is also important for energy supply as well as raw materials for hormonal biosynthesis in plants. A good plant preparation by promoting a good carbohydrate accumulation in plants and leaves is more advantage. Plant managements, e.g. proper pruning, sufficient water and nutrient supply are suggested especially prior to cool day season.