Chapter 5

Effects of low night temperature on the changes in some endogenous hormones and other biochemical substances in *Curcuma alismatifolia* Gagnep.

5.1 Introduction

Temperature is an important seasonal cue that enables plant to predict and consequently prevent the adverse effects of environmental change. Plant responses to temperature are two folds. Temperature signals can act as a stimulus to control the timing of developmental transitions and to enhance tolerance to future temperature extremes (Franklin, 2009). Recent studies have also demonstrated temperature signals in regulation of germination, plant architecture, flowering and enhancements of freezing tolerance by evoking changes in hormone levels (Franklin, 2009) and other biochemical substances. Although temperatures in tropical regions do not vary drastically throughout the year, certain plants nevertheless show sensitivity to these conditions in their growth, possibly via changes in the endogenous hormones and their biochemical substances.

It was found that the temperature affected the growth and flower quality of *Curcuma alismatifolia* plant, especially during off-season production under low night

temperature (average night temperature at 18°C) conditions (Hongpakdee *et al.*, 2009). Previous studies found the evidence that changes in temperature induced fluctuations in the levels of plant hormones, such as abscisic acid in in *Dendrobium* cv. 'Second Love' (Campos and Kerbauy, 2004) and bulbous plants (Okubo and Uemoto, 1981), cytokinin in a *Phalaenopsis* hybrid (Chou *et al.*, 2000), and auxin in *Arabidopsis* (Thingnaes *et al.*, 2003). Plant nutrition was also found to be affected by the temperature, e.g., N uptake in *Medicago sativa* (Noquet *et al.*, 2003). A number of reports had also indicated that the uptake of N both as NH_4^+ and NO_3^-, was temperature dependent, and the rate of uptake was reduced under low temperature (Mengel and Kirkby, 1987). Generally, low temperature caused a considerable increase in the accumulation of free sugar contents (Guy *et al.*, 1992; Kumar *et al.*, 2004; Sasaki *et al.*, 1996). The relationship between low temperature and amino acid metabolism has been investigated (Sagisaka, 1974). The effects of temperature factors on growth processes must be known in order to understand their effects on plants as a whole. Cell growth, especially cell elongation, has the high Q10 value, which indicates that it depends on a chemical processes rather than physical ones (Went, 1953).

Therefore, this research was focused on the effects of low night temperature on certain aspects of plant biochemistry, i.e., the concentration of endogenous hormones, free sugar contents, free amino acids and nutritional status.

5.2 Materials and methods

5.2.1 Plant materials and experimental conditions

Stubbed rhizomes of *C. alismatifolia* cv.'Chiang Mai Pink' were planted in the same technique and condition as described in **'Chapter 4'** . At sprouting, they were moved to experimental plots. Plants were grown in a controlled environmental growth chamber (Conther phytotron climate simulator) for 24 weeks at 30/24°C day temperature/night temperature (DT/NT) as to control high night temperature (HNT) and 30/18°C for low night temperature (LNT). The day length, relative humidity (% RH) and light intensity were maintained at 13 h day and 11h night, 70-80% RH and 270 μmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF), respectively. The irradiance was provided by cool daylight fluorescent tubes. There was no barrier between the light source and plants. The cultural practices and other maintenances on plant samples were the same as explained in the experiment of **'Chapter 4'**

Plant growth, endogenous hormones and other biochemical substances were analyzed as described in **'Chapter 4'**.

5.2.2 Statistical analysis

Collected data were analyzed for statistical significance using Statistic 8 analytical software (SXW Tallahassee, FL, USA). Student's *t*-test was used to determine significant differences between the means in growth (with ten replicates per treatment), hormone and other biochemical substances (three replicates, three plants per replicate) parameters.

5.3 Results

5.3.1 Plant growth and development

Compared with control treatment (HNT), the LNT treatment did not influence the plant height or the total leaf area. However, it decreased the leaf dry weight from 7.6 g to 5.2 g and reduced the number of shoots per cluster from 10.8 to 7.4. However, plants grown with LNT had more number of leaves than those grown with control (HNT) (Table 5.1).

Table 5.1 Plant height (cm), total leaf area (cm^2), number of leaves, leaf DW (g) and number of shoots per cluster of *Curcuma* plants grown under 30/24°C as high night temperature (HNT) and 30/18°C as low night temperature (LNT) conditions, at the flowering stage (12 WAP)

Treatment	Height	Total leaf area	No. of leaves	Leaf DW	Shoots per cluster
HNT (30/24°C)	53.5 ± 2.3	424 ± 21.2	3.3 ± 0.2	7.6 ± 0.2	10.8 ± 1.3
LNT (30/18°C)	54.3 ± 1.4	413 ± 35.3	3.8 ± 0.2	5.2 ± 0.7	7.4 ± 0.5
t-test at $p < 0.05$	ns	ns	*	*	*

Data were means ± SE ($n=10$). *; significant, ns = not significant

LNT treatment significantly reduced the flower spike length and flower stalk length to 50% and 55% of the lengths observed with HNT treatment, respectively (Table 5.2). It delayed flowering by more than one week (from 64 to 73 days after planting) and reduced the percentage of flowering from 100 to 60%. However, the numbers of green bracts and coma bracts were not statistically different between treatments (Fig. 5.1).

Table 5.2 Flower quality of *Curcuma* plants grown under 30/24°C as high night temperature (HNT) and 30/18°C as low night temperature (LNT) conditions, at the flowering stage (12 WAP)

Treatment	Flower quality					
	Spike length (cm)	Flower stalk length (cm)	No. of green bracts	No. of coma bracts	Days to flower	% Flowering
HNT(30/24°C)	12.7 ± 0.7	51.2 ± 1.5	9.0 ± 0.3	12.8 ± 0.5	64.4 ±2.0	100
LNT(30/18°C)	6.3 ± 0.3	28.3 ± 0.8	8.8 ± 0.5	11.5 ± 0.5	73.0 ±2.2	60
t-test						
at $p < 0.05$	*	*	ns	ns	*	-

Data are means ± SE ($n=10$). *; significant, ns = not significant

Figure 5.1 Comparison of flower quality of *C. alismatifolia* grown under (**A**) high night temperature (HNT 30/24°C) and (**B**) low night temperature (LNT 30/18°C) at the flowering stage (12 week after planting: WAP).

Neither the yields of new rhizomes (total number, fresh weight, dry weight and diameter) nor the yields of new storage roots (total number, fresh weight, dry weight, diameter and length) of plants were significantly affected by LNT treatment at the dormancy stage (24 WAP) (Table 5.3).

Table 5.3 Rhizome yields of *Curcuma* plants grown under different night temperature conditions at the dormancy stage (24 WAP)

Yields	Treatment		*t*-test[1/]
	HNT (30/24°C)	LNT (30/18°C)	
New rhizomes			
total numbers	4.2 ± 0.8	5.4 ± 1.2	ns
fresh weight (g)	12.9 ± 3.6	19.1 ± 4.4	ns
dry weight (g)	4.9 ± 0.6	5.1 ± 0.7	ns
diameter (cm)	1.7 ± 0.2	1.8 ± 0.1	ns
New storage roots			
total numbers	2.4 ± 0.8	1.6 ± 1.0	ns
fresh weight (g)	8.9 ± 3.5	6.4 ± 3.2	ns
dry weight (g)	2.2 ± 0.2	1.7± 0.3	ns
diameter (cm)	0.7 ± 0.3	0.3 ± 0.3	ns
length (cm)	4.8 ± 0.6	5.2 ± 0.5	ns
Contractile roots			
total number	-	-	-
length (cm)	-	-	-

[1/] Significant by Student's *t*-test at $p<0.05$

Data were means ± SE ($n=10$)

5.3.2 Changes in endogenous hormones

ABA concentrations

At 12 WAP, the concentrations of ABA and *t*-ZR were analyzed in leaves, rhizomes and storage roots of *C. alismatifolia*. The results showed that the ABA levels in all plant parts (leaf, rhizomes and storage roots) did not significantly different between HNT and LNT conditions (Fig. 5.2A). However, it seemed that the highest endogenous ABA levels (1,200 ng gDW^{-1}) tended to be found in leaves under the HNT condition, but they were only about 900 ng gDW^{-1} in leaves under the LNT condition. In contrast, the ABA levels in rhizomes and storage roots tended to increase by LNT treatment from 140 to 200 ng $gDW^{-1}$and from 300 to 500 ng gDW^{-1}, respectively as compared with those under HNT condition (Fig. 5.2A).

t-ZR concentrations

Endogenous *t*-ZR levels in leaves did not seem to differ significantly between treatments. It was interesting to note that *t*-ZR significantly accumulated in rhizomes and storage roots under the LNT condition. Its level in rhizomes increased from 100 to 400 ng gDW^{-1}, and its level in storage roots increased from 230 to 530 ng gDW^{-1} (Fig. 5.2B). It was speculated that the fully expanded leaf was the organ that contained the lowest level of *t*-ZR under all conditions (Fig. 5.2B).

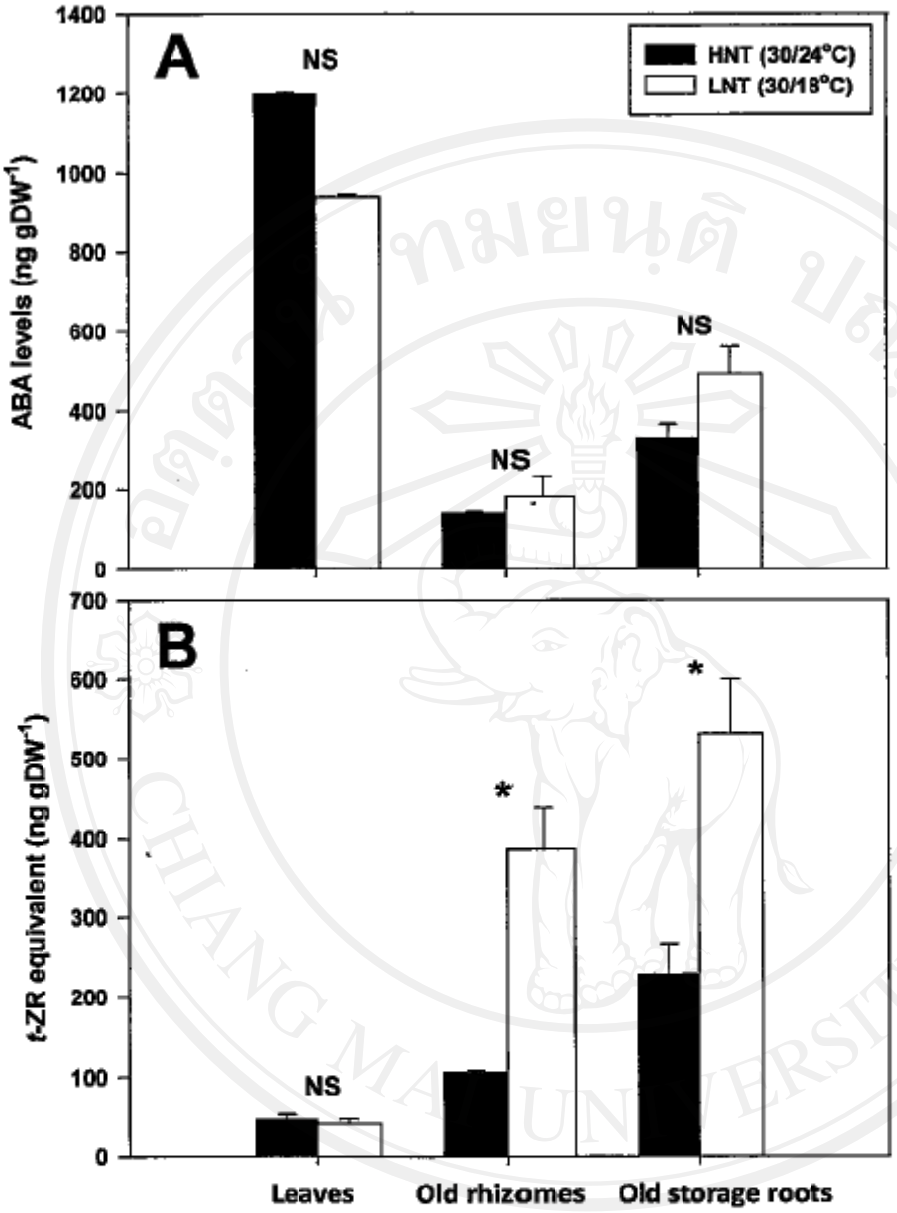

Figure 5.2 Comparison of ABA (**A**) and *t*-ZR (**B**) levels in leaves, old rhizomes and old storage roots of *C. alismatifolia* grown under different day temperature/night temperature (DT/NT) conditions between high night temperature (HNT 30/24°C) and low night temperature (LNT 30/18°C). Hormone determinations were conducted at the flowering stage (12 week after planting: WAP). Values are means ± SE (*n=3*).

5.3.3 Free sugars content

At flowering stage of *C. alismatifolia*, free sugars contents were analyzed in leaves, old rhizomes and storage roots. The result showed that LNT treatment significantly reduced glucose and fructose concentrations in leaves, while the sucrose concentrations were negligible to detect in both night temperature treatments (Fig. 5.3A).

All free sugars contents (glucose, fructose and sucrose) in old rhizomes were not significantly different between the two treatments (Fig. 5.3B).

Both glucose and fructose concentrations in old storage roots under LNT condition (4.25 and 2.95 mg gDW^{-1}) were significantly lower than those under HNT condition (8.70 and 6.89 mg gDW^{-1}). However, the large amount of sucrose was found in old storage roots, but was not different between treatments (116.33 and 126.89 mg gDW^{-1}, respectively) (Fig. 5.3).

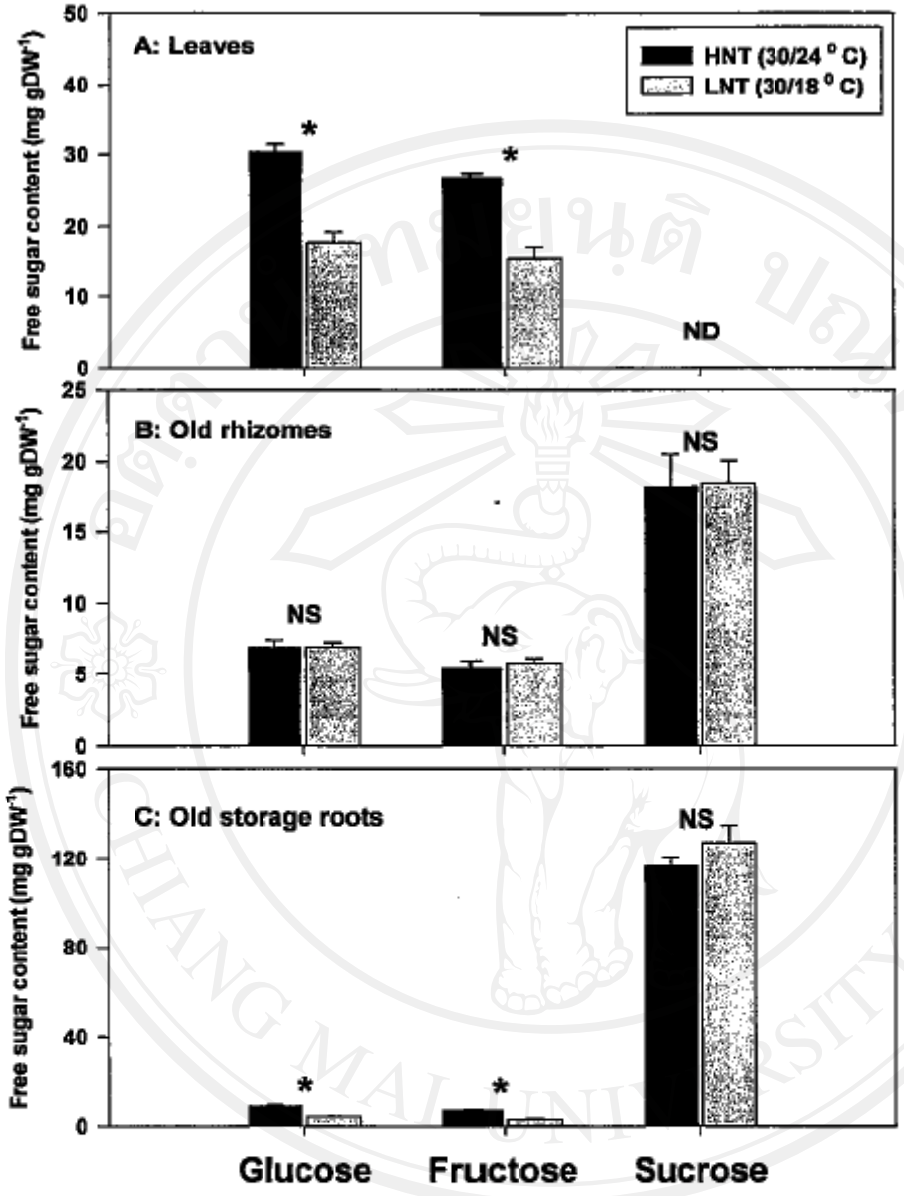

Figure 5.3 Free sugar contents (mg gDW^{-1}) in leaves (**A**), old rhizomes (**B**) and old storage roots (**C**) of *C. alismatifolia* under the different night temperature conditions, between high night temperature (HNT) 30/24° C (black bar) and low night temperature (LNT) 30/18° C (grey bar). *, Significantly different at $p < 0.05$ at the same stage. NS; not significantly different. ND; Not detected.

5.3.4 Amide-N (total free amino acids concentration)

At flowering stage, LNT conditions significantly decreased total amino acids in leaves and old storage roots from 6.31 to 4.39 and 4.38 to 2.46 mg gDW^{-1}, respectively, as compared with HNT conditions. The highest concentration was found in leaves under HNT condition at 6.31 mg gDW^{-1}. However, its concentration in old rhizomes was not affected by night temperature treatment (Table 5.4).

Table 5.4 Total free amino acids concentration in various organs of *Curcuma* plants grown under 30/24°C as high night temperature (HNT) and 30/18°C as low night temperature (LNT) conditions, at the flowering stage (12 WAP)

Treatment	Total free amino acids concentration (mg gDW^{-1})		
	Leaves	Old rhizomes	Old storage roots
HNT (30/24°C)	6.31 ± 0.35	5.27 ± 0.79	4.38 ± 0.12
LNT (30/18°C)	4.39 ± 0.25	6.26 ± 0.33	2.46 ± 0.11
t-test	*	ns	*

Data were means ($n = 4$) ± SE.

*; significant, ns = not significant

5.3.5 Nutrient contents

Low night temperature reduced the N content in the aboveground parts of *C. alismatifolia* plant at the flowering stage. The highest N content was found in leaves (162 mg plant^{-1}) of plants under HNT conditions. On the other hand, there was no difference in the N contents in rhizomes of plants grown under HNT or LNT conditions (15.4 and 13.3 mg plant^{-1}, respectively). However, N contents in storage roots grown under LNT were higher than those grown under HNT (Table 5.5).

In contrast, P contents showed higher accumulation in leaves under LNT (102 mg plant^{-1}) than under HNT conditions (35.7 mg plant^{-1}). P contents were significantly different in rhizomes, with a lower P content (4.7 mg plant^{-1}) resulting from LNT conditions. Nevertheless, there was no difference in the P content of storage roots of plants grown under HNT or LNT conditions (12.2 and 10.6 mg plant^{-1}, respectively) (Table 5.5).

Under HNT conditions, leaves were the organ that contained the highest K contents (415 mg per plant), and they were greater than those in leaves under LNT conditions (281 mg per plant). However, the K content in rhizomes under LNT condition (19.3 mg per plant) was slightly higher than that under HNT condition (15.3 mg per plant). Neither HNT nor LNT conditions affected the K contents of storage roots (Table 5.5).

Table 5.5 Nitrogen (N), phosphorus (P) and potassium (K) contents (mg plant^{-1}) in leaves, rhizomes and storage roots of *Curcuma* plants grown under different day temperature/night temperature conditions, HNT (30/24°C) and LNT (30/18°C), at the flowering stage (12 WAP)

Organs	Nutrient content (mg plant^{-1})					
	N		P		K	
	HNT	LNT	HNT	LNT	HNT	LNT
Aboveground						
Leaves	162 ± 5.4	88.7 ± 6.2*	35.7 ± 5.8	102 ± 3.1*	415 ± 36.7	281 ± 2.3*
Underground						
Old rhizomes	15.4 ± 1.5	13.3 ± 0.7^{ns}	14.2 ± 1.4	4.7 ± 1.3*	15.3 ± 0.6	19.3 ± 1.4*
Old storage roots	19.9 ± 2.4	29.2 ± 0.6*	12.2 ± 3.0	10.6 ± 2.5^{ns}	115 ± 9.1	97.3 ± 5.3^{ns}
Total	197 ± 7.1	131 ± 5.8*	62.1 ± 6.8	117 ± 1.7*	546 ± 31.6	397 ± 22.8*

*Significant by Student's *t*-test at $p<0.05$

Data are means ± SE ($n=3$)

5.4 Discussion

The results showed that the LNT treatment appeared to have a greater influence on reproductive growth (spike length, flower stalk length and percent flowering) than on vegetative growth, except for the dry weight of leaves and the number of shoots per a cluster (Table 5.1 and 5.2). Plants grown under LNT had more leaves, but produced less leaf dry weight and fewer shoots per cluster. HNT treated plants accumulated more biomass and had a greater number of shoots per a cluster. Similar result was found in potatoes which grown under high temperature condition produced more dry matter content and sugars content in tuber (Kumar *et al.*, 2003). In general, plants produced the maximum growth when they were exposed to a day temperature about 5.5 to 8.0°C higher than the night temperature (Arizona Cooperative Extension, 2009).

Partitioning of biomass in leaves, stem and roots was markedly affected by day and night temperatures (Blackshaw and Entz, 1995). Roberts (1943) suggested that the temperature during the night, rather than during the day, largely determined the response of the plant to temperature. In *Phalaenopsis*, it had been shown that the leaves incorporated much higher quantities of CO_2 under higher temperature conditions (32/28°C) than lower temperature conditions (21/16°C), resulting in the increase in leaf growth (Guo and Lee, 2006). Thus, a similar response might be explained in the case of *Curcuma* plant.

The LNT treatment decreased the spike length, flower stalk length and percentage of flowering as compared with HNT treatment (Table 5.2). Other species also showed a negative response in their reproductive processes, when they were

exposed to low temperatures. Low temperature induced flower abortion in chickpea (Nayyar et al., 2005), inhibited pollen function in peppers (Shaked *et al.*, 2004) and delayed flowering in chrysanthemum (Fukai *et al.*, 2000), lisianthus (Takezaki *et al.*, 2000), calla lily (Naor and Kigel, 2002) and cyclamen (Oh *et al.*, 2008). This might be due to the insufficient supply of assimilate (glucose and fructose concentrations) in leaves and old storage roots under the LNT treatment (Fig. 5.3). Considering the observation above, a night temperature of 18°C might be too low for these plants to reach their maximum rates of growth and development. Thingnaes *et al.* (2003) found that temperature affected the levels of phytohormones in *Arabidopsis,* especially an increase in night temperature decreased the auxin levels in stem tissue and might have reduced cell volume (epidermal and pith cell length), resulting in reduced stem elongation. In addition, LNT treatment was reported to decrease the levels of ABA in the leaves of *Dendrobium* cv. 'Second Love' (Campos and Kerbauy, 2004).

At the flowering stage, the underground organs of *C. alismatifolia* began to swell, forming into food reserve structures (Chidburee, 2008). In this experiment, LNT might have stimulated the earlier-harvested rhizomes (data not shown) without affecting the size and yields of new rhizomes or new storage roots (Table 5.3). The acceleration of assimilate mobilization to underground organs would be caused by the increase of *t*-ZR in underground tissue (Fig 5.2B) and was induced by the changes in day and night temperatures (Blackshaw and Entz, 1995).

Generally, plants were thought to synthesize ABA in their leaves and sometimes in their roots (Davies and Jones, 1992). It was also found that other species of plants increased the ABA levels under the low temperatures, as chilling conditions

or during acclimation for cold stress resistance (Welling *et al.*, 1997; Yamazaki *et al.*, 1999). The accumulations of ABA could be considered to be a physiological response of the adaptive process (Zeevaart, 1999). In this research, statistical analysis showed that LNT treatment did not affect ABA levels in all plant parts (leaves, rhizomes and storage roots). However, when plant was grown under off-season condition in Thailand (LNT combined with SDL), ABA level was significantly increased (Hongpakdee *et al.*, 2009). This indicated that endogenous ABA level in *C. alismatifolia* was affected by unfavorable conditions both LNT and SDL, but LNT was a mild effect as compared with than that of SDL.

Root tips were the major sites of CK biosynthesis (Letham, 1978). *t*-ZR, the translocated form of CK (Gordon *et al.*, 1974; Hewett and Wareing, 1973; Letham, 1974), was usually synthesized in the roots (Kuroha *et al.*, 2002; Letham, 1994) and it was also involved in root formation by controlling the rate of meristematic cell differentiation (Dello Ioio, 2007; Kyozuka, 2007; Werner *et al.*, 2003). This hormone might control sink strength by activating the expression of gene implicated in assimilation partitioning (Roitsch and Ehneβ, 2000). Therefore, the increase in *t*-ZR concentration in rhizomes and storage roots might occur as a result of its synthesis in the roots and exported to the mother rhizome to promote new storage organs. Cool night temperatures had been reported to favor tuberization in potatoes by inducing earlier tuber initiation (Slater, 1968) and to produce faster bulbing in onions (Steer, 1980). Hence, the new rhizomes of *Curcuma* plant which grown under LNT condition could be harvested faster than HNT conditions.

Mineral nutrient analysis showed the decrease in the N and K concentrations in *Curcuma* plants subjected to the LNT condition. The LNT condition decreased N, P and K contents in all underground parts of the plant (new rhizomes and storage roots), except for a slight increase in the N content of storage roots. However, in aboveground parts of the plant (leaves), N and K contents were reduced by LNT. The temperature of the root zone was known to alter plant growth, as well as, nutrient uptake by roots (Engels *et al.*, 1992; Mozafar *et al.*, 1993). In particular, low temperature had been reported to lower the uptake rates of both NH_4^+ and NO_3^- (Mengel and Kirkby, 1987). Low soil temperature limited the shoot and root growth, and nutrient and water uptake. As a result, low temperature reduced overall plant growth and tended to increase carbon allocation to the roots, because nutrient and water uptake were reduced (Lambers *et al.*, 1995; Li *et al.*, 1994).

Under low soil temperatures, shoot demand seemed to control uptake and translocation of N and K but not of P (Engels *et al.*, 1992). On the other hand, the uptake of P was usually more severely inhibited by low soil temperatures than the uptake of other nutrients (Bravo-F and Uribe, 1981; Engels *et al.*, 1992). A previous report showed that cool soil temperature at 15°C reduced K^+ influx from outside of the root of *Zea mays* L., resulting in less root growth (Ching and Barbers, 1979). However, result from this experiment here showed that LNT treatment did not influence size and yields of the underground parts.

Nevertheless, the decreases in aboveground biomass accumulation (leaf dry weight) and number of shoots per cluster under LNT treatment could probably be related to the decreases in total N and K uptake. The decrease in spike length, flower

stalk length and percentage of flowering under LNT conditions might be related to the lower N and K contents, as due to the important role of N in protein and RNA synthesis during flower induction periods and the involvement of K in meristematic growth.

LNT treatment decreased the total free amino acids concentration in leaves and old storage roots under LNT treatment, causing a considerable decrease in N contents of whole plants, since the excess N supply appeared to enhanced protein accumulation in rhizomes of *Curcuma* (Ohtake *et al.*, 2006).

5.5 Conclusion

From this experiment, it could be concluded that the LNT condition induced hormonal and other biochemical changes in *C. alismatifolia*. ABA and *t*-ZR were affected differently by the temperature treatments. Although, LNT did not affect the growth inhibitors (ABA) in all plant parts, it increased growth promoters (*t*-ZR) in underground organs. LNT treatment seemed to be reducing free sugars contents (fructose and glucose), and total free amino acids concentration in leaves and old storage roots. In addition, this condition also decreased the N and P contents of rhizomes and the N and K contents it leaves. Furthermore, these changes were partially leading plant to beneficial aboveground growth and better flower quality, but were not affecting all rhizomes yields. It was speculated that the response of ABA might be the limitation in stimulating the formation of new rhizomes in *C. alismatifolia*. Further works are needed to determine the modes of action of this factor.