Chapter 4

Effects of day length on the changes in some endogenous hormones and other biochemical substances in *Curcuma alismatifolia* Gagnep.

4.1 Introduction

A myriad of important physiological processes in horticultural crops are regulated by plant hormones and other biochemical substances; as well as, the changes in environmental factors. Environmental factors, in particular photoperiods, affect the growth and development of many plant species (Le Nard and De Hertogh, 1993). Although photoperiod factor 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 biochemical substances.

Curcuma alismatifolia Gagnep. should be classified as quantitative long day plants, since the long day condition, with supplemental lighting, promoted the flowering (Hagiladi *et al.*, 1997a). It was found that photoperiods affected the growth and flower quality of this plant, especially during off-season (OS) production under short day (average 11 h). This condition was speculated to induce ABA accumulation

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in various organs and led to depress shoot growth and flower quality (Hongpakdee *et al.*, 2009).

Generally, off-season cropping of *C. alismatifolia* in Thailand was usually practiced for avoiding surpluses of the regular season cut flower or rhizome production and brought about increasing profit for growers. However, they also faced the different growth and yields quality in both seasonal cropping which could not produce the better than another in off-season times. Nevertheless, an increase of day length by giving 2 h of night break treatment could improve the flower quality, but produced less storage organs (Ruamrungsri *et al.*, 2007).

Previous studies found some evidences that changes in photoperiods could induce fluctuations in the levels of plant hormones, such as abscisic acid in bulbous plants (Okubo and Uemoto, 1981), cytokinin in a *Phalaenopsis* hybrid (Chou *et al.*, 2000), and zeatin in *Dendrobium* cv. 'Second Love' (Campos and Kerbauy, 2004), and that brought to the various growth and development in plants. There was an evidence that the uptake of plant nutrients (except K and B) in both roots and shoots of *Zea mays* L. was reduced by extending the day length from 6 to 18 h at a given root zone temperature (Mozafar *et al.*, 1993). Previous investigations demonstrated that the partitioning of photosynthate from source to sink was affected by photoperiods (Demers *et al.*, 1998; Logendra and Janes, 1992; Logendra *et al.*, 1990; Lorenzen and Ewing, 1992). The effects of photoperiod factors on growth processes must be known in order to clearly understand their effects on plants as a whole.

Therefore, the aim of this study was to provide specific information on the changes in some physico-chemicals; including the endogenous hormones, free sugars

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contents, free amino acids contents and nutrient status; in leaves, old rhizomes and storage roots of *C. alismatifolia* grown under short day length condition for further research to be establishing the appreciate technique for off-season production. These data may explain, partially, the roles of ABA, *t*-ZR, and other biochemical substances in relation to shoot depression and storage organs promotion in the previous study.

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4.2 Materials and methods

4.2.1 Plant materials and experimental conditions

Rhizomes of *C. alismatifolia* cv.'Chiang Mai Pink' with diameters of 1.8-2.5 cm and 4 storage roots were obtained from an exporter (Ubonrat Garden, Chiang Mai, Thailand) and stored at 15°C, 70% RH until use. All rhizomes were planted in 6 X 12 inch plastic bags filled with mixed medium (soil, rice husks and rice husk charcoal in a 1:1:1 ratio by volume). At sprouting, they were moved to experimental plots.

Plants were grown in a growth chamber for 24 weeks with 13 h day length and 11 h night length as control (LDL) or 11 h day length and 13 h night length (SDL). Other environmental factors were set as the temperature was 30/24°C (DT/NT), 70-80% RH and 270 μmol $m^{-2}s^{-1}$ PPF, respectively. The irradiance was provided by cool daylight fluorescent tubes. There was no barrier between the light source and plants.

In this experiment, plants were daily irrigated with fixed volume of 450 mL water throughout the growth periods, and each plant was supplied with 7.5 g of 16-16-16 (N-P_2O_5-K_2O) fertilizer since the first leaves were fully expanded through the flowering stage and with 13-13-21 (N-P_2O_5-K_2O) fertilizer since the flowering stage through the rhizome-forming stage, twice a month.

Plant growth; in terms of plant height, total leaf area, number of leaves, leaf dry weight (DW), number of shoots per cluster, flower quality and rhizome yield, were measured. Endogenous hormones (ABA and CK) , free sugars contents (glucose, fructose and sucrose), total free amino acids concentration and plant nutrients (N, P

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and K) were analyzed in leaves, rhizomes and storage roots at the flowering stage (12 weeks after planting: WAP). The analytical methods were the same as in **'chapter 3'**.

4.2.2 Statistical analysis

Data were analyzed for statistical significance using Statistic 8 analytical software (SXW Tallahassee, FL, USA). The student's *t*-test was used to determine significant differences between the means in growth (with ten replicates per treatment), hormones, free sugars contents, free amino acids concentration and nutrient contents (three replicates, three plants per replicate) parameters.

4.3 Results

4.3.1 Plant growth and development

Day length did not affect total leaf area, number of leaves or leaf dry weight of *C. alismatifolia* plants. The SDL of 11 h significantly increased plant height from 50.0 to 56.7 cm but decreased the number of shoots per cluster from 7.2 to 4.0 when compared with LDL (Table 4.1) treatment.

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Table 4.1 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 different day length conditions, at the flowering stage (12 WAP)

Treatment	Height	Total leaf area	No. of leaves	Leaf DW	Shoots per cluster
LDL (13 h)	50.0 ± 0.9	269 ± 47.6	4.8 ± 0.2	6.7 ± 0.3	7.2 ± 0.8
SDL (11 h)	56.7 ± 1.5	312 ± 29.6	5.1 ± 0.3	6.7 ± 0.3	4.0 ± 0.0
t-test	*	ns	ns	ns	*

Data were means ± SE (*n=10*), LDL = Long day length, SDL = Short day length

*; significant, ns = not significant

Surprisingly, the flower stalk length, number of green bracts, number of coma bracts and number of days to flowering were not affected by the SDL condition. However, the spike length of SDL plants were decreased from 11.7 to 8.9 cm and the percentage of flowering were reduced to 60% as compared with those of the LDL treated plants (Table 4.2).

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Table 4.2 Flower quality of *Curcuma* plants grown under different day length 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
LDL (13 h)	11.7 ± 0.4	43.9 ± 1.4	7.0 ± 0.0	10.0 ± 0.3	71.6 ± 1.0	100
SDL (11 h)	8.9 ± 0.1	45.3 ± 0.6	7.5 ± 0.2	5.5 ± 0.8	80.5 ± 3.5	60
t-test at $p < 0.05$	*	ns	ns	ns	ns	-

Data were means ± SE ($n=10$), LDL = Long day length, SDL = Short day length

*; significant, ns = not significant

At the dormancy stage, the yields of new rhizomes and new storage roots of *Curcuma* plants were affected by SDL treatment. It was found that plants grown under SDL conditions had produced lower yields of new rhizomes than those grown under LDL. The SDL condition also decreased the total number (from 7.8 to 3.4 rhizomes per cluster), fresh weight (from 21.0 to 9.2 g), dry weight (from 5.3 to 3.5 g), and size of new rhizomes as compared with the LDL condition (Table 4.3 and Fig. 4.1).

In contrast, the SDL condition dilivered greater yields of new storage roots than the LDL condition. Plants grown under SDL had significantly increased total numbers (from 4.6 to 12.6 storage roots per rhizome), fresh weight (from 31.9 to 48.4 g) and dry weight (from 2.7 to 13.4 g) of new storage roots. The SDL condition led plants to have larger new storage roots (1.7 cm in diameter); however, these storage

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roots were shorter in length with no contractile root as compared with that of the LDL (Table 4.3 and Fig. 4.1).

Table 4.3 Rhizome yields of *Curcuma* plants grown under different night temperature and day length conditions, at the dormancy stage (24 WAP)

Yields	Treatment		*t*-test[1/]
	LDL (13 h)	SDL (11 h)	
New rhizomes			
total numbers	7.8 ± 0.6	3.4 ± 0.5	*
fresh weight (g)	21.0 ± 2.2	9.2 ± 1.0	*
dry weight (g)	5.3 ± 0.5	3.5 ± 0.4	*
diameter (cm)	1.8 ± 0.3	1.4 ± 0.0	*
New storage roots			
total numbers	4.6 ± 0.4	12.6 ± 1.4	*
fresh weight (g)	31.9 ± 5.2	48.4 ± 4.9	*
dry weight (g)	2.7 ± 0.3	13.4 ± 2.2	*
diameter (cm)	1.0 ± 0.2	1.7 ± 0.2	*
length (cm)	5.5 ± 0.5	2.9 ± 0.3	*
Contractile roots			
total number	15.4 ± 1.6	-	-
length (cm)	14.2 ± 4.3	-	-

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

Data were means ± SE ($n=10$)

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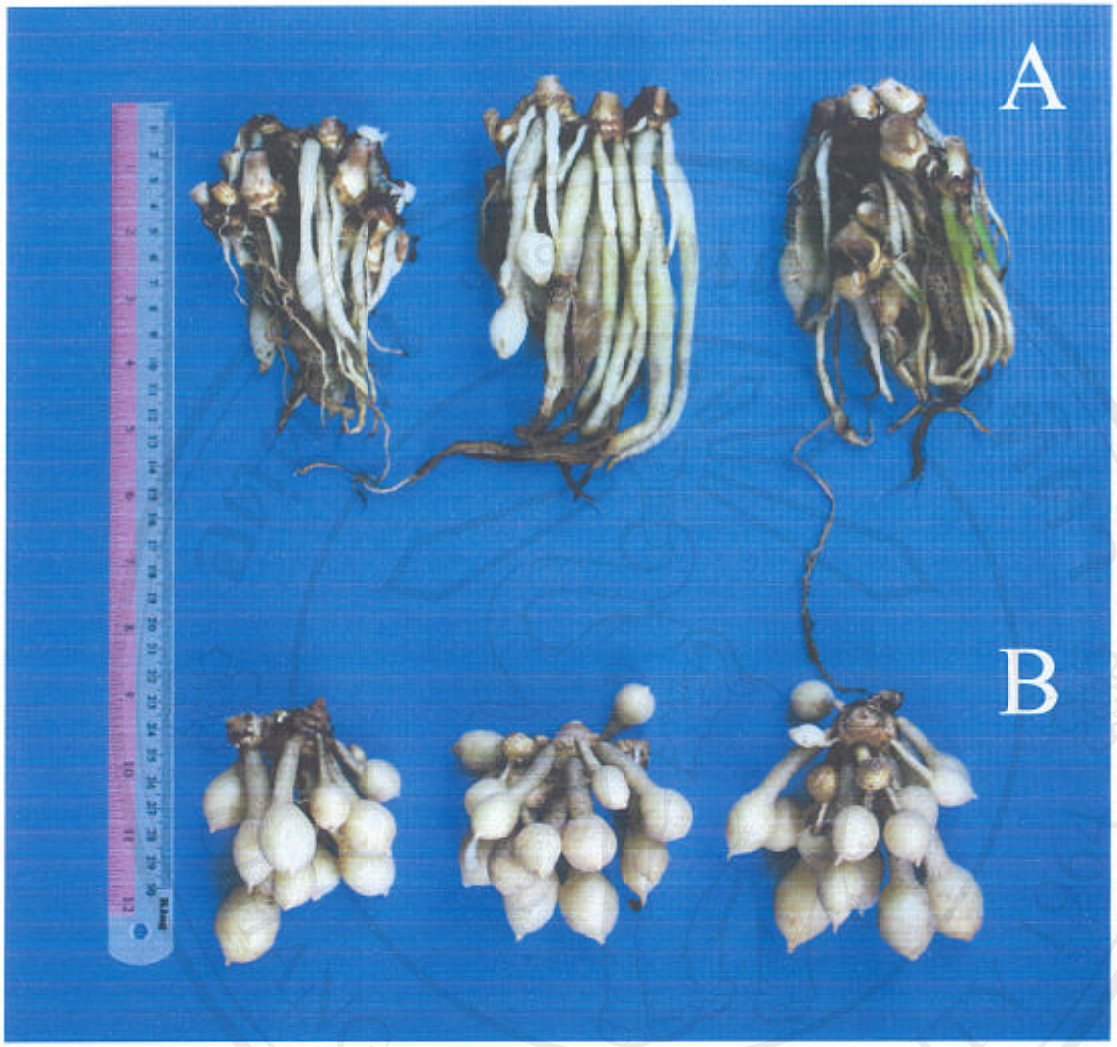

Figure 4.1 Comparison of new rhizome yields and new storage root yields of *C. alismatifolia* grown under LDL (13 h) conditions (A) or SDL (11 h) conditions (B) at the dormancy stage (24 weeks after planting: WAP).

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4.3.2 Changes in endogenous hormones

ABA concentrations

At the flowering stage, the highest free endogenous ABA levels accumulated in leaves under the SDL condition (800 ng gDW^{-1}), and they were significantly higher than those under the LDL condition (600 ng gDW^{-1}). In rhizomes and storage roots, no significant difference in ABA levels was observed between the two treatments (240 and 250 ng gDW^{-1} and 330 and 220 ng gDW^{-1}, respectively). (Fig. 4.2A).

t-ZR concentrations

High levels of endogenous *t*-ZR were found in rhizomes (1,200 ng gDW^{-1}) and storage roots (2,000 ng gDW^{-1}) under the SDL condition. In both treatments, leaves contained less endogenous *t*-ZR (47 and 42 ng gDW^{-1}). Under the SDL condition, rhizomes and (especially) storage roots had significant increases in *t*-ZR levels from 900 to 1,200 and 1,200 to 2,000 ng gDW^{-1}, respectively (Fig. 4.2B).

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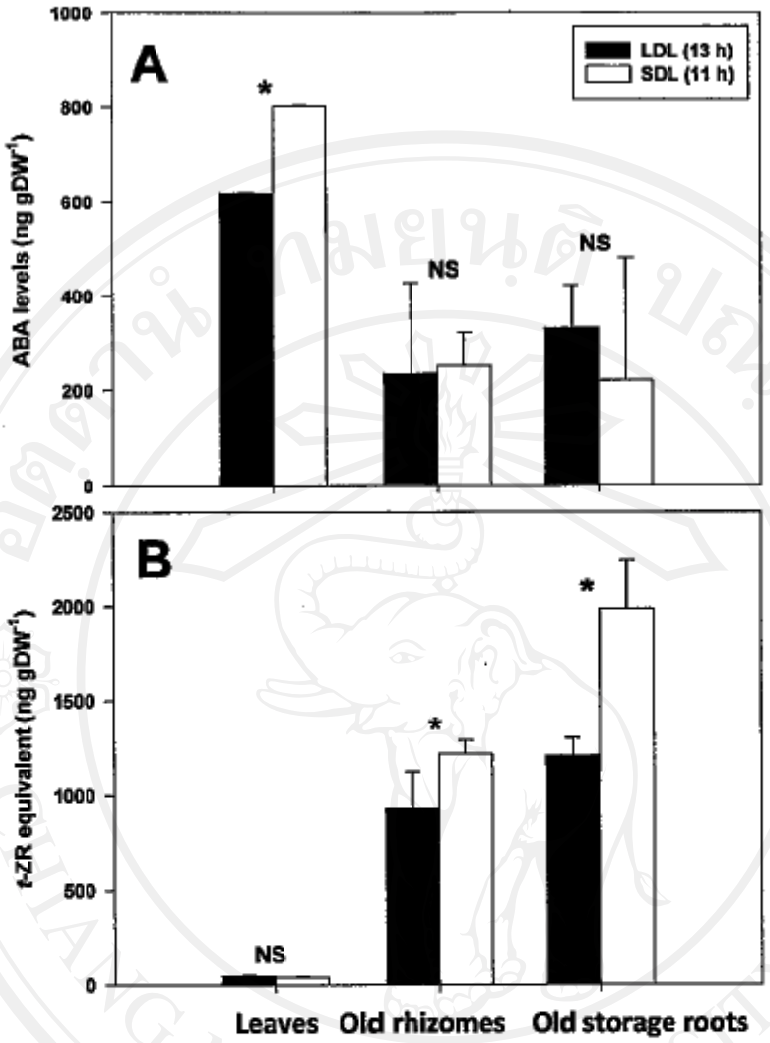

Figure 4.2 Comparison of ABA (**A**) and *t* –ZR (**B**) levels in leaves, old rhizomes and old storage roots of *C. alismatifolia* grown under long day length (LDL 13 h) and short day length (SDL 11 h) conditions. Hormone determinations were conducted at the flowering stage (12 week after planting: WAP). Values were mean ± SE (*n*=*3*).

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4.3.3 Free sugars contents

At flowering stage (12 WAP) free sugars contents were analyzed in leaves, old rhizomes and old storage roots. The result showed that neither glucose nor fructose concentrations in leaves were significantly differed among LDL and SDL condition, while the sucrose concentrations in both day lengths were negligible to detect (Fig. 4.3A).

SDL treatment significantly decreased glucose, fructose and sucrose concentrations in all underground organs (old rhizomes and storage roots) (Fig. 4.3B and C). The highest sucrose concentration was found in old rhizomes under LDL condition (28.36 mg gDW^{-1}) (Fig. 4.3).

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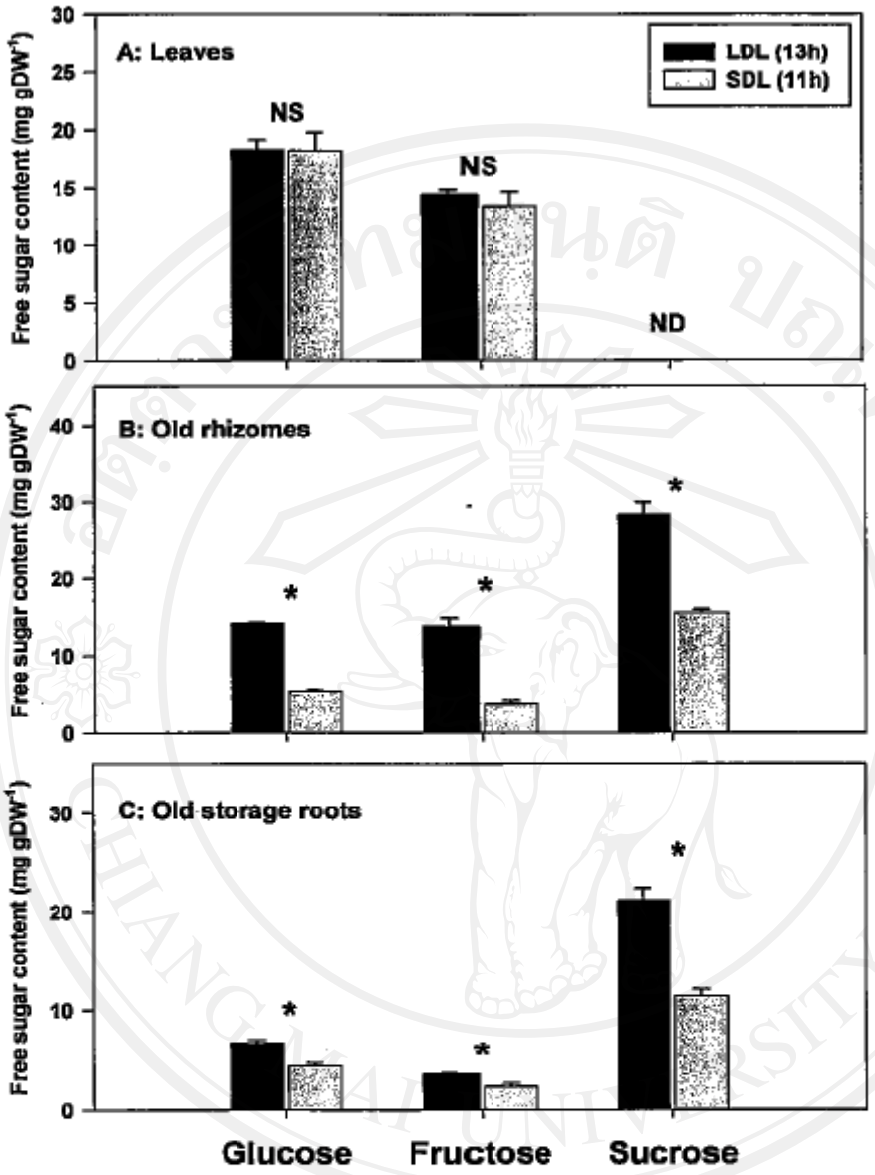

Figure 4.3 Free sugar content (mg gDW^{-1}) in leaves (**A**), old rhizomes (**B**) and storage roots (**C**) of *C. alismatifolia* grown under long day length (LDL) 13 h (black bar) and short day length (SDL) 11 h (grey bar) conditions. *, Significantly different at $p < 0.05$ at the same stage. NS; not significantly different. ND; Not detected.

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4.3.4 Amide-N (total free amino acids concentration)

SDL condition caused significantly higher total free amino acids in leaves, old rhizomes and storage roots (7.10, 12.20 and 5.37 mg gDW^{-1}) than the LDL condition (5.25, 7.50 and 3.37 mg gDW^{-1}). The highest concentration was found in old rhizomes under SDL condition (12.20 mg gDW^{-1}) at flowering stage of *C. alismatifolia* (Table 4.4).

Table 4.4 Total free amino acids concentration of 80% ethanol soluble in various organs of *Curcuma* plants grown under long day length (LDL) 13 h and short day length (SDL) 11 h at flowering stage (12 WAP).

Treatment	Total free amino acids (mg gDW^{-1})		
	Leaves	Old rhizomes	Old storage roots
LDL (13 h)	5.25 ± 0.24	7.50 ± 0.60	3.37 ± 0.14
SDL (11 h)	7.10 ± 0.36	12.20 ±0.36	5.37 ± 0.46
t-test	*	*	*

Data were means ($n = 4$) ± SE.

*; significant, ns = not significant

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4.3.5 Nutrient contents

The SDL condition decreased N contents in underground parts of the *Curcuma* plant. The high N content was observed in leaves under the LDL condition (163 mg plant^{-1}), which was not significantly different from that observed under the SDL condition (142 mg plant^{-1}) (Table 4.5).

P contents were significantly increased in leaves and rhizomes under the LDL condition (109 and 140 mg plant^{-1}) when compared with those under the SDL condition (38.4 and 52.0 mg plant^{-1}). On the other hand, storage roots under the LDL condition contained less P (8.1 mg plant^{-1}) than those under the SDL condition (29.6 mg per plant) (Table 4.5).

Leaves and rhizomes of plants grown under the LDL condition had higher K contents (423 and 170 mg plant^{-1}) than those of plants grown under the SDL condition (335 and 94.8 mg plant^{-1}). However, the K contents in storage roots did not differ between the two treatments (164 and 141 mg plant^{-1}) (Table 4.5).

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Table 4.5 Nitrogen (N), phosphorus (P) and potassium (K) contents (mg plant^{-1}) in leaves, rhizomes and storage roots of *Curcuma* plants grown under different day length conditions LDL (13 h) and SDL (11 h), at the flowering stage (12 WAP)

Organs	Nutrient content (mg plant^{-1})					
	N		P		K	
	LDL	SDL	LDL	SDL	LDL	SDL
Aboveground						
Leaves	163.0 ± 7.2	142.0 ± 9.3^{ns}	109.0 ± 5.6	38.4 ± 6.5*	423.0 ± 17.8	335.0 ± 23.5*
Underground						
Old rhizomes	128.0 ± 5.5	109.0 ± 3.3*	140.0 ± 11.6	52.0 ± 6.3*	170.0 ± 19.1	94.8 ± 14.5*
Old storage roots	40.2 ± 1.6	29.6 ± 2.0*	8.1 ± 0.8	29.6 ± 2.5*	164.0 ± 14.2	141.0 ± 10.3^{ns}
Total	331.0 ± 8.9	280.0 ± 10.9*	256.0 ± 13.7	120.0 ± 10.5*	757.0 ± 28.8	570.0 ± 39.9*

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

Values were means ± SE ($n=3$)

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4.4 Discussion

Curcuma plants grown under SDL were taller than those under LDL, consistent with previous observations (Changjeraja *et al.*, 2008), but the former produced less leaf mass (Table 4.1). The decrease in shoot number per a cluster under SDL treatment indicated that there were insufficient assimilates to promote growth, although total leaf area, leaf number and leaf dry weight were not different between treatments (Table 4.1).

SDL treatment affected some growth parameters of the plants, especially spike length and percentage of flowering. These effects were similar to those observed in plants grown in the off-season production without supplemental lighting (Ruamrungsri *et al.*, 2007).

When flowers were produced, assimilates were translocated to promote the development of flowers (Weaver and Johnson, 1985). The reduced spike length might have been caused by the insufficient assimilates (free sugars contents in underground organs) found under SDL treatment and possibly related to the changes in endogenous hormones. It is well known that auxins, CKs and GAs promote the mobilization of assimilates by creating metabolic sinks and may create competition among sinks for assimilates (Goldschmidt and Huber, 1992). The percentage of flowering was also reduced, consistent with the report of Chidburee (2008), this was caused by flower abortion, which was probably influenced by the short day treatment.

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The results of this experiment also showed that the levels of ABA in the leaves of plants grown under LDL conditions were lower than those in the leaves of plants grown under SDL conditions. However, the *t*-ZR levels in leaves did not seem to be

different (Fig. 4.2B). The levels of *t*-ZR in rhizomes and storage roots significantly increased under SDL conditions (Fig. 4.2B). The increase of ABA in leaves could inhibit spike elongation, since exogenous ABA had been reported to inhibit shoot growth and stem elongation in tulips (Saniewski *et al.*, 1990), and shoot elongation and floral development in Dutch iris (Doss *et al.*, 1983). In onions, the injection of ABA into bulbs delayed sprouting (Abdel-Rahman and F.M.R., 1974) and inhibited shoot growth in onion culture (Mahotiere *et al.*, 1976). While the increase of *t*-ZR in old rhizomes and storage roots stimulated the mobilization of assimilates from the old underground organs (source) to the new one (strength sink) rather than the inflorescence. This resulted in the decreased of free sugars contents in source under SDL condition.

It was interesting that photoperiod affected the size of rhizomes and storage roots more than the day/night temperatures. The size of new rhizomes under short day length (11 h) treatment was smaller than that under long day length (13 h) treatment. On the other hand, storage roots were enlarged by SDL treatment. Comparable results were found in a previous study (Changjeraja *et al.*, 2008). *Solanum* species represented an ideal model for studies of photoperiodic control of both flowering and tuber formation (Machackova *et al.*, 1998). Their flowering responses were different from that of *C. alismatifolia*, a quantitative long day plant, in which day length did not absolutely inhibit flowering (Hagiladi *et al.*, 1997a; Ruamrungsri *et al.*, 2007), although it was similarly found that SDL conditions promoted storage root formation. Light was found to directly regulate the biosynthesis of active GAs (Oh *et al.*, 2006; Seo *et al.*, 2006) and ethylene (Steed *et al.*, 2004) and to control the degradation of

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ABA passing through the transduction pathway of phytochrome signal both at the tissue and organism levels (Kraepiel *et al.*, 1994).

The relationship between plant hormones and stem swelling has rarely been reported. In potatoes, low GAs and high ABA levels stimulated tuber induction (Machackova *et al.*, 1998). Wang *et al.* (2006) found a positive correlation between dry tuberous root yields and the concentration of ZR and DHZR in sweet potatoes, and suggested that they played a key role in determining whether the adventitious roots could transform into tuberous roots and the rate of tuberous root thickening by the activity of the secondary cambium. The *t*-ZR level was about 30% higher in tissue induced to tuberize than in non-induced tissues (Mauk and Langille, 1978). The same study also showed that *t*-ZR levels in old rhizomes and storage roots under short day length conditions were 40% higher than those found under long day length conditions, and that day length might be related to the swelling of new storage roots.

These results here supported the previous report that supplemental lighting to provide long day conditions during off-season production may cause a decrease in ABA levels in leaves and bring about the lengthening of the spike. The decrease of new storage roots number found in the night break treatment (Ruamrungsri *et al.*, 2007) might have been resulted from the reduction in *t*-ZR in old rhizomes and storage roots caused by LDL treatment.

It has previously been reported that day length can induce fluctuations of some phytohormones (Campos and Kerbauy, 2004; Chou *et al.*, 2000; Okubo and Uemoto, 1981; Yamazaki *et al.*, 1999), and this may be partly due to its effect on nutrient uptake by plant roots. There was an evidence that the uptake of plant nutrients (except

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K and B) in both roots and shoots of *Zea mays* L. was reduced by extending the day length from 6 to 18 h at a given root zone temperature (Mozafar *et al.*, 1993). In this experiment, the opposite result was found; SDL treatment decreased the total N, P and K contents, especially the N contents in the underground parts of the plant (rhizomes and storage roots). In potatoes, tuber induction was favored by short day length, cool temperature, the balance of some phytohormones (low GAs, high ABA and high CKs) and a low rate of nitrogen fertilizer (Krauss, 1985). One factor affecting this phenomenon was the low rate of N supply: Ohtake *et al.* (2006) found that low N supply brought about an increase in the dry weight of storage roots of *C. alismatifolia.* Therefore, this favorable condition might stimulate the swelling of storage roots. The mechanism by which low levels of N promoted storage root formation was not known, but the correlation of a decrease in N, a decrease in GAs and an increase in ABA had been reported (Krauss, 1985) as a set of hormonal changes that favored tuber formation. Also, a similar response in which low levels of N promoted the swelling organs might be expected in the case of this study.

Utilization of N was observed in total free amino acids concentration. The increased of total free amino acids concentration was found in all plant parts under SDL condition. This was in consistent with the result found in tobacco *(Nicotiana tabacum)* in which SDL decreased in total free amino acids and protein content (Matt *et al.*, 1998).

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4.5 Conclusion

The basic information on the changes in endogenous hormones and other biochemical substances related to photoperiods could provide a better understanding of this plant and it would be useful for developing an appropriate technique for off-season production. From the experiments described above, it could be concluded that the distinct environmental factors, as SDL, induced hormonal and other biochemical changes. ABA and *t*-ZR were affected differently by the treatments. SDL treatment induced high ABA in the aboveground organs and *t*-ZR in underground organs. SDL treatment seemed to reduce free sugars content in underground organs and N, P and K accumulation overall, except for P accumulation in storage roots. The increased total free amino acids concentrations were apparently caused by the SDL treatment. Also, these changes were partially related to the depression of some aboveground growth, but promotion of storage organs by the photoperiod factors. Further experiments are needed to determine the modes of action of this factor.

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