Chapter 5

Effect of day length on growth and rhizome formation of *Curcuma alismatifolia*

5.1 Introduction

C. alismatifolia is an herbaceous perennial in Zingiberaceae family and it produces stubbed rhizomes with spherical shape storage roots (Burch *et al*., 1987). The buds start to emerge after a dormancy period and the shoots (pseudo-stem) subsequently grow and then floral anthesis occurs within 2-3 months after shoot emergence. Several new rhizomes with storage roots are formed at the basal part of the pseudo-stem, and they go dormant in winter. In Thailand, seasonal production of curcuma starts from April to May by planting dry stored rhizomes. Flowering occurs from July to August during the rainy season in Thailand, when the average temperature is 27-28^{o}C, 12-13 hrs of daytime duration and 70-80% RH. Then plants undergo dormancy in November to December when the temperature is approximately 30/16^{o}C (max/min), 10 hrs of the average sunshine duration and 65-70% air RH. Rhizomes are usually harvested in December to January and is stored at room temperature with good air-ventilation. Standard size of rhizome for export is about 2 cm in diameter with 4-5 storage roots attached (Department of Agricultural Extension, 2005). Ruamrungsri *et al*. (2001) reported that both rhizome and storage roots reserved nitrogen and carbohydrates, but the rhizomes are the principal organ for nitrogen reserve, and the storage roots are the major organ for carbohydrate storage. At the beginning of dormancy, the new rhizomes stored about 90 mg N, 548 mg starch and 104 mg of soluble sugar per rhizome, and storage roots reserved about 50 mg of N, 2,000 mg of starch and 470 mg of soluble sugar per rhizome. Sufficient supply of N was important for flower quality and rhizome production. Nitrogen absorption activity was the highest from the two expanded leaf stages, at about

6 weeks after planting (WAP), to the first floret opening stage, at around 9 WAP (Ruamrungsri *et al*., 2006).

Photoperiod and light intensity are the major factors which affect the growth and development of these plants. Light not only supplies the energy upon which plant life is based, but via various photomorphogenetic mechanisms, it also plays an important role in directing its energy along the various possible metabolic pathways (Wassink and Stolwijk, 1956). Kuehny *et al.* (2002) reported that plant height of *C. alismatifolia* increased as photoperiod increased to 20 hrs. The number of leaves also increased at 16 and 20 hrs of photoperiod. Perception of day length is accomplished by leaves and one or more stimuli are then translocated to the responsive regions for growth and development induction (Thomas and Vince-Prue, 1997). Shanmugand and Muthuwamy (1974) found that long day condition increased N, K, Ca, Mg and carbohydrate concentration in leaves of *Chrsysanthemum indicum*.

It is well known that growth of plants is affected by both internal and external e.g. by light, water, nutrients, hormones etc. The regulation mechanisms of these factors are only partially understood (Lawlor and Lawlor, 1995). As light is an important environmental factor for flowering and dormancy of *C. alismatifolia*, it is important to understand how growth and development are affected by day length especially under short day and low light intensity. Short day length may cause the changes in vegetative and reproductive growth and the morphology of the plant thrsough changes of phytohormone levels. Also short day may restrict the carbohydrate supply due to short period of photosynthesis or lower photosynthetic activity.

5.2 Materials and methods

5.2.1 Plant materials

Rhizomes of *Curcuma alismatifolia* cv. Chiang Mai Pink with the diameter of 2.5 cm and 4-5 storage roots were grown in black plastic pots containing sand: rice husk: rice husk charcoal at the ratio of 1:1:1 (Fig. 5.1a). After new shoot and roots emerged, plants were transferred to controlled rooms with three day length treatments, i.e.7, 10 and 13 hrs by using artificial light sources (36 watts of cool-white fluorescent tubes, 405-812 nm), 60 µmol $m^{-2}s^{-1}$ PAR at 27±2 ^{o}C, 70-80% RH until harvest

(Fig. 5.1b). Another group of rhizome was grown in the field under natural conditions as control (25-35^{o}C, 12-13 hrs of sunshine and 28-71% air RH) at Lampang Agriculture Research and Training, Rajamangala University of Technology Lanna, Lampang province. Each plant was supplied with 100 ml of nutrient solution that comprised of 200 mg N, 50 mg P, 65 mg Ca, 200 mg K, 20 mg Mg, 0.22 mg B, 0.54 mg Mn, 0.26 mg Zn, 0.04 mg Mo and 0.45 mg Fe per liter. The plant was supplied with nutrient solution three times a week.

5.2.2 Data collection

5.2.2.1 Recording of vegetative growth

The growth and development of plants in terms of plant height (cm), and diameter of pseudostem (cm) and number of leaves per plant were measured every two weeks. The number of new rhizome per cluster, diameter of new rhizome, weight of new rhizome, number of storage roots per rhizome, the size of storage root (length and diameter) and weight of storage roots were collected when harvested.

a

b

Figure 5.1 Plant materials (a) grown in a growth chamber (b).

5.2.2.2 Photosynthetic efficiency

The measuring of photosynthetic rate was operated on the fist mature leaf. Ten plants were selected and measured at 9:00 and 10:00 o'clock by using a handy leaf cuvette connected to the leaf chamber analyser type LCA-4 (Halma Group, Germany). Photosynthetic rate (µmol CO_2 $m^{-2}s^{-1}$) was recorded.

5.2.2.3 Chlorophyll fluorescence

The fist leaf form shoot apex was selected and measured for chlorophyll fluorescence by Plant Efficiency Analyser (PEA) (Hansatech, Germany) at 9:00 and 10:00 o'clock from 10 plants (Fig. 5.2).

Figure 5.2 Measurement of chlorophyll fluorescence.

5.2.2.4 Measurement of total chlorophyll content

The same leaves as used for photosynthetic rate measurement were analyzed at 10:00 and 11:00 o'clock using chlorophyll meter (Model SPAD-502, Minota, Japan) in order to determine foliar leaf chlorophyll concentration *in vivo*. A calibration of the SPAD value was made based on the chlorophyll analysis method.

Figure 5.3 Measurement of chlorophyll content using SPAD

To produce a calibration curve, two fully mature leaves were randomly selected per one sample. The measurement was made on 30 samples (60 leaves). Each leaf measurement of SPAD value was made at 4 positions on the mid-region of the leaf blade. The four values were then averaged to obtain a single SPAD value for each leaf. For chlorophyll extraction, four leaf disks per leaft were punched out from the leaf blade at the same area with chlorophyll SPAD measurement using 0.3 cm^2 paper puncher. Chlorophyll was eluted from these disks by dipping them in DMF (N,N-Dimethyformamide). Samples were stored in the dark at room temperature for 48 hrs. Absorbance readings were obtained by using Spectrophotometer (at 647 and 664 nm in 1 cm cuvette, and chlorophyll concentration was calculated according to the following formula and expressed as g m^{-2} of leaf:

Chl_a = $(-2.99A_{647} + 12.64A_{664}) * Vol / (X * Area * 100)$

Chl_b = $(23.26A_{647} + 15.60A_{664}) * Vol / (X * Area * 100)$

Chl_{total} = $(20.27A_{647} + 7.04A_{664}) * Vol / (X * Area * 100)$

Chl_a =Volume of chlorophyll a, g m^{-2}

Chl_b =Volume of chlorophyll b, g m^{-2}

Chl_{total} = Volume of total chlorophyll, g m^{-2}

A_{647} =Value of absorb at 647 nm

A_{664} =Value of absorb at 664 nm

Vol =Volume of DMF, ml

X =Ratio of dilute solution (when reading absorb more than 0.8)

Area = Area of leaf to selection, cm^2

100 = number of multiply convert unit g cm^{-2} to g m^{-2}

The procedure was described by Moran (1982).

5.2.3 Plant analysis

Two parts i.e., rhizome and storage roots were sampled at every weekly interval from each treatment to analyses for total nonstructural carbohydrates, reducing sugars, starch and total soluble sugars (see appendix A-E). Free sugars (glucose, fructose and sucrose) in leaves were determined by Gas Chrsomatograph (see appendix F). Total nitrogen in rhizome and storage roots was analyzed by the modified Kjeldahl method (Ohyama *et al*., 1985) (see appendix G). Total amino acid contents also were determined by the ninhydrin method (Takahashi *et al*., 1993) (see appendix H).

5.2.4 Gene expression during the rhizome formation by differential display (DD RT-PCR) (see appendix I)

New rhizome was weekly sampled from 15 to 23 WAP from each treatment to determine gene expression using DD RT-PCT.

5.2.5 Statistical analysis

The experimental design in this research was Completely Randomized Design (CRD) with 10 replications per treatment (1 plant per replication). A statistical

analysis of data was made by using Statistica version 7 for Windows. Means separated by Least Significance Different test (LSD).

5.3 Results

5.3.1 Vegetative growth

Growth of *C. alismatifolia* grown under different day length (7, 10 and 13 hrs) compared with natural environment conditions (control) were shown in Fig 5.4. Plant height of the 7, 10 and 13 hrs day length treatments were greater than control (Fig. 5.4a, Fig. 5.6 and Fig. 5.7) and they were no significant difference between the 7, 10 and 13 hrs. Life cycle of plant under 13 hrs day length, from planting to dormancy. It took about 24 weeks and it was longer than the other.

The number of leaves per plant was not significantly different among treatments (Fig. 5.4b). The average number of leaves per plant in all treatments was 3.07-3.69 leaves per plant.

Diameters of pseudo-stem in all treatments increased from planting until 7 WAP. Then, those of the 7, 10 and 13 hrs of day length decreased and were different from those that grew in natural light (Fig. 5.4c). Under natural light conditions, the average diameter of a pseudo-stem was 1.24 cm at 16 WAP.

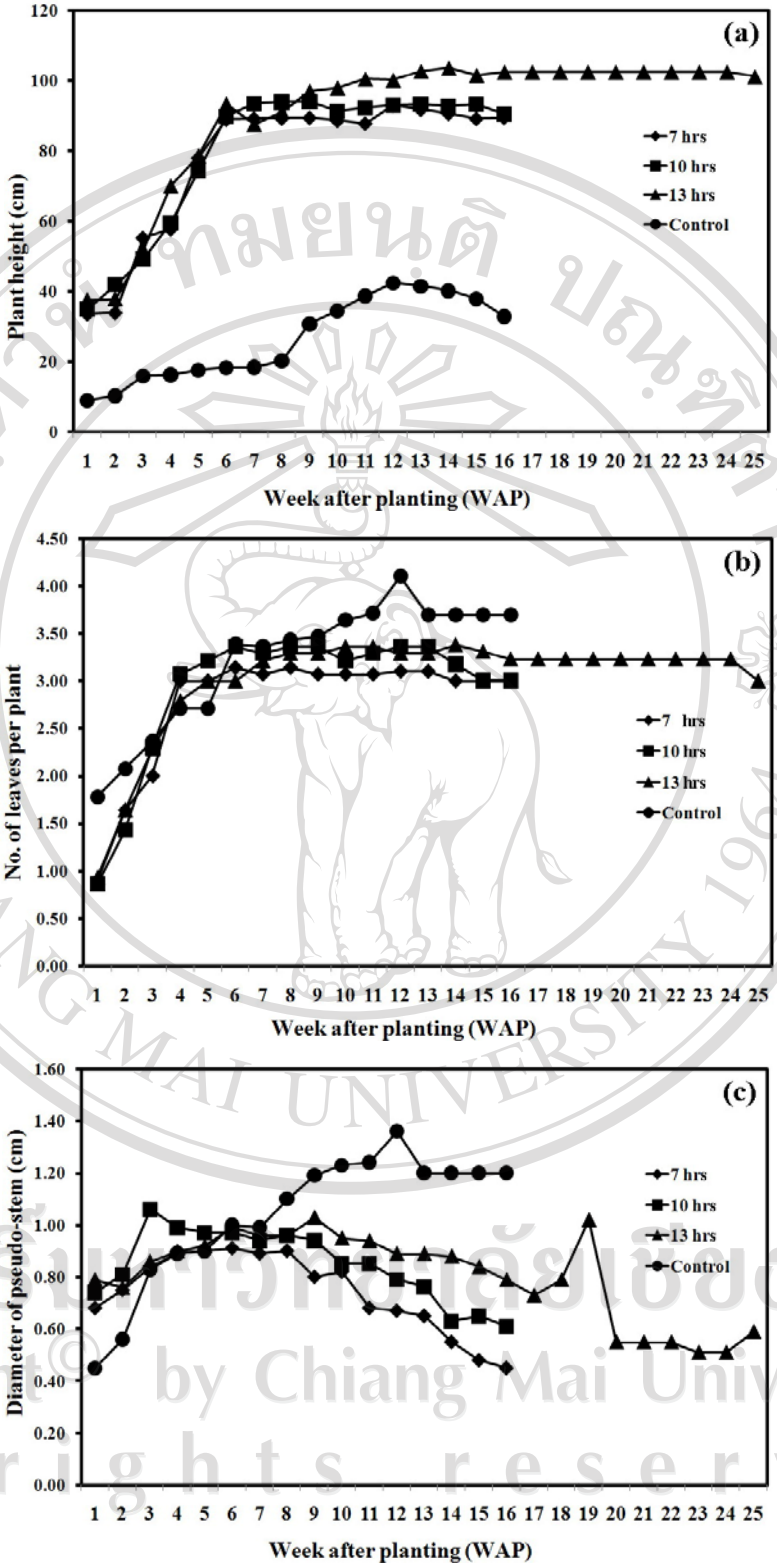


Figure 5.4 Plants height (a), number of leaves per plant (b) and diameter of pseudo-stem (c) under different day length conditions (7, 10, 13 hrs and control).

a b

Figure 5.5 Plants under different day length conditions (a: at 7, 10 and 13 hrs) compared with natural environmental condition (b) at 4 WAP.

a b

Figure 5.6 Plant height and pseudo-stem sizes under different day length conditions (a: at 7, 10 and 13 hrs) compared with natural environmental condition (b) at 12 WAP.

Table 5.1 shows that height of plants grown under 13 hrs was higher than 103 cm and it was significantly difference from those of 7 hrs and control (90.67 and 32.89 cm, respectively). Seven hours treatment caused a decrease in the diameter of pseudo-stem (0.55 cm) compared with the others. The number of leaves per plant grown under control (3.69 leaves per plant) was higher than other treatments (3.00, 3.00 and 3.33 leaves under 7, 10 and 13 hrs, respectively).

Table 5.1 Effect of day lengths (7, 10, 13 hrs and control) on plant height, diameter of pseudo-stem, number of leaves per plant cultured at 15 WAP.

Day length (hrs)	Height (cm) [1/]	Diameter of pseudo-stem (cm) [1/]	No. of leaves per plant [1/]
7	90.67±34.30b	0.55±0.02b	3.00±0.00b
10	96.00±14.20ab	0.68±0.03ab	3.00±0.00b
13	103.00±28.20a	0.82±0.19a	3.33±1.33ab
control	32.89±17.18c	1.20±0.23a	3.69±0.45a

[1/]: Mean within the same column followed by different letter were significantly different between treatments at $P<0.05$

The result also shows that plants grown under 7, 10 and 13 hrs could flower at 6 WAP (Fig. 5.7a and b) but floral buds abortion occurred under 7 hrs (Fig. 5.8a and b). However, floral bud could normally develop under 13 hrs (Fig.5.8a)

a b c

Figure 5.7 Inflorescence of plants under 7 hrs (a and b) compared with controlled treatment (c) at 6 WAP. Bar = 1 cm.

a b

Figure 5.8 Inflorescences under 13 hrs of day length (a) at 8 WAP compared with controlled treatment (b) at 10 WAP.

5.3.2 Development of rhizome and storage roots

At 5 WAP, plants started to develop new rhizome and storage roots. Those organs enlarged and the terminal of some contractile roots was swollen to store food reserve (Fig. 5.9).

Figure 5.10 shows new rhizome at harvest. Number of new rhizomes per cluster and number of storage roots controlled was higher than those of 7, 10 and 13 hrs. Moreover, the length of these storage roots was shorter than controlled ones.

a b

Figure 5.9 Development of rhizomes and storage roots under 10 hrs day length condition at 5 WAP (a) and 7 WAP (b).

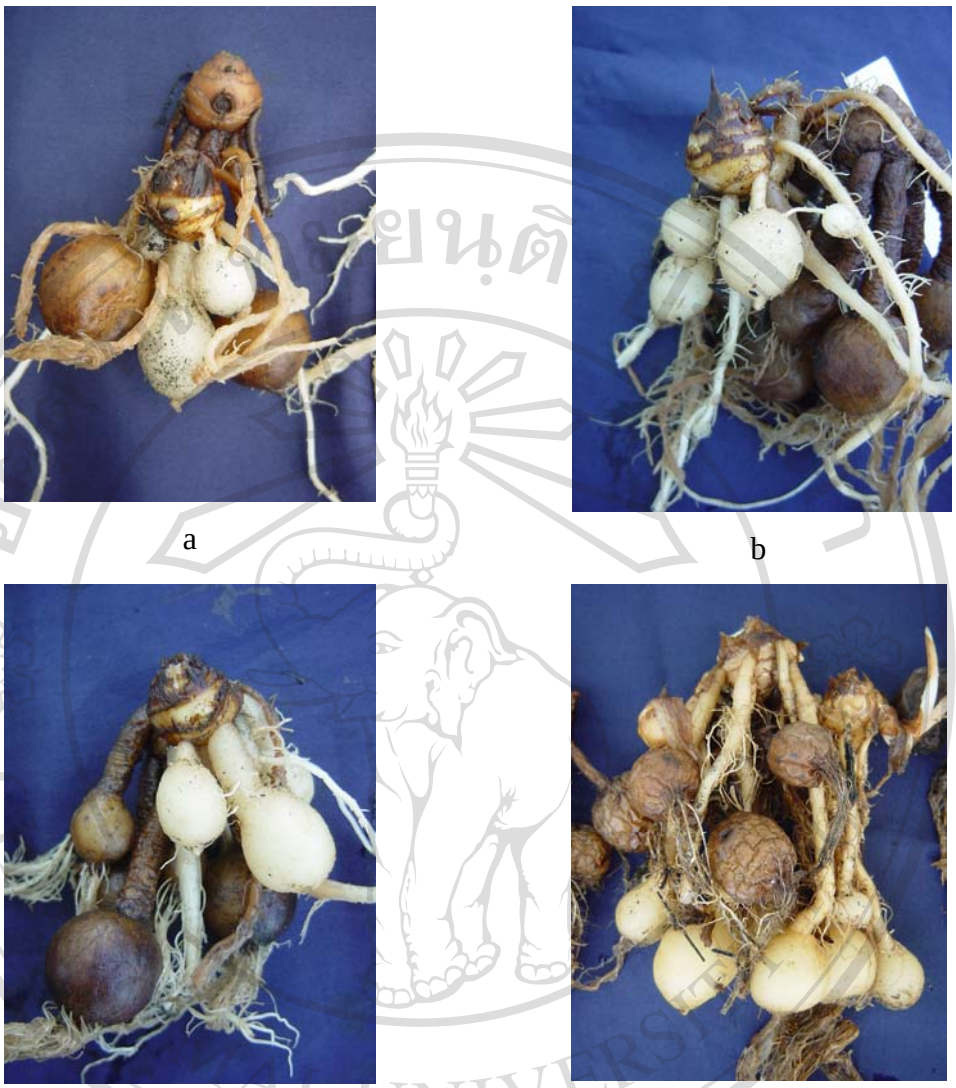


Figure 5.10 Rhizomes and storage roots grown under 7 (a), 10 (b), 13 hrs (c) day length conditions compared with controlled treatment (d) at harvest.

Quality and yield of rhizome were determined at harvest. The results showed that the number of new rhizomes per cluster under different day length (7, 10 and 13 hrs) (1 new rhizome per cluster) was lower than that under natural conditions (3.67 new rhizomes per cluster) (Table 5.2). Diameter of new rhizomes under 13 hrs day length and natural conditions was 2.40 and 2.30 cm respectively, which was higher than those of 7 and 10 hrs; 1.80 and 1.60 cm respectively. However, fresh and dry weight of new rhizome was not significantly different.

The number of storage roots per rhizome under controlled treatment (7.67 storage roots per rhizome) was higher than other treatments (Table 5.2). The results also indicated that shorter day length at 7 and 10 hrs decreased the number of storage roots per rhizome and length of storage roots compared with 13 hrs. However, the diameter of storage roots seemed to be the same. Day length did not affect fresh and dry weight of storage roots because it was not significantly different among those of 7, 10 and 13 hrs. Growing plants under controlled treatment gave the higher degree of fresh and dry weight (17.55 and 2.20 g, respectively).

Table 5.2 Effect of day length on number and quality of rhizomes and storage roots at harvest.

New rhizome				
Day length (hrs)	No. of new rhizome per cluster [1/]	Diameter of new rhizome (cm) [1/]	Weight of new rhizome (g)	
			Fresh [1/]	Dry [1/]
7	1.00±0 b	1.80±0.29ab	5.43±0.34ab	1.48±0.21ab
10	1.00±0b	1.60±0.17b	6.82±0.71a	1.40±0.71ab
13	1.00±0b	2.40±0.38a	6.97±0.81a	1.66±0.61a
Control	3.67±0.13a	2.30±0.89a	6.48±0.65a	1.66±0.78a

Storage roots					
Day length (hrs)	No. of storage roots per rhizome [1/]	Size of storage roots (cm)		Weight of storage roots (g)	
		Length [1/]	Diameter [1/]	Fresh [1/]	Dry [1/]
7	2.87±0.75c	4.50±0.24b	1.40±0.17ab	6.03±1.72b	0.08±0.03b
10	2.93±0.91c	3.66±0.53c	1.50±0.28a	6.52±2.34b	0.06±0.06b
13	3.50±0.83b	5.94±0.61ab	1.12±0.93b	8.94±4.83b	0.11±0.10b
Control	7.67±0.34a	7.89±0.54a	1.60±0.21a	17.55±1.45a	2.20±0.76a

[1/] Mean within the same column followed by different letter were significantly different between treatments at $P<0.05$

5.3.3 Photosynthetic efficiency

Photosynthetic rate (*Pn*) was measured at the fully matured first leaf at 3, 4 and 5 WAP (Fig. 5.11a). The results showed that the *Pn* value of the plants under 13 hrs of day length had a relatively higher activity at 50-55 µmol $m^{-2}s^{-1}$ than the others at 3 WAP and it was not significantly different from the controlled at 4 WAP and 7 hrs at 5 WAP (Fig. 5.11a). The *Pn* values of the shorter day length at 7 hrs was not significantly different from 10 hrs day length.

5.3.4 Chlorophyll fluorescence

The values of chlorophyll fluorescence (*Fv/Fm*) indicate the potential photosynthetic activity. The results show that the range of values were relatively constant between 0.81 and 0.84 at 3, 4 and 5 WAP under 10 and 13 hrs day length. The *Fv/Fm* ratio was a little bit decreased from 0.84 to 0.81 at 5 WAP under 7 hrs day length (Fig. 5.11b).

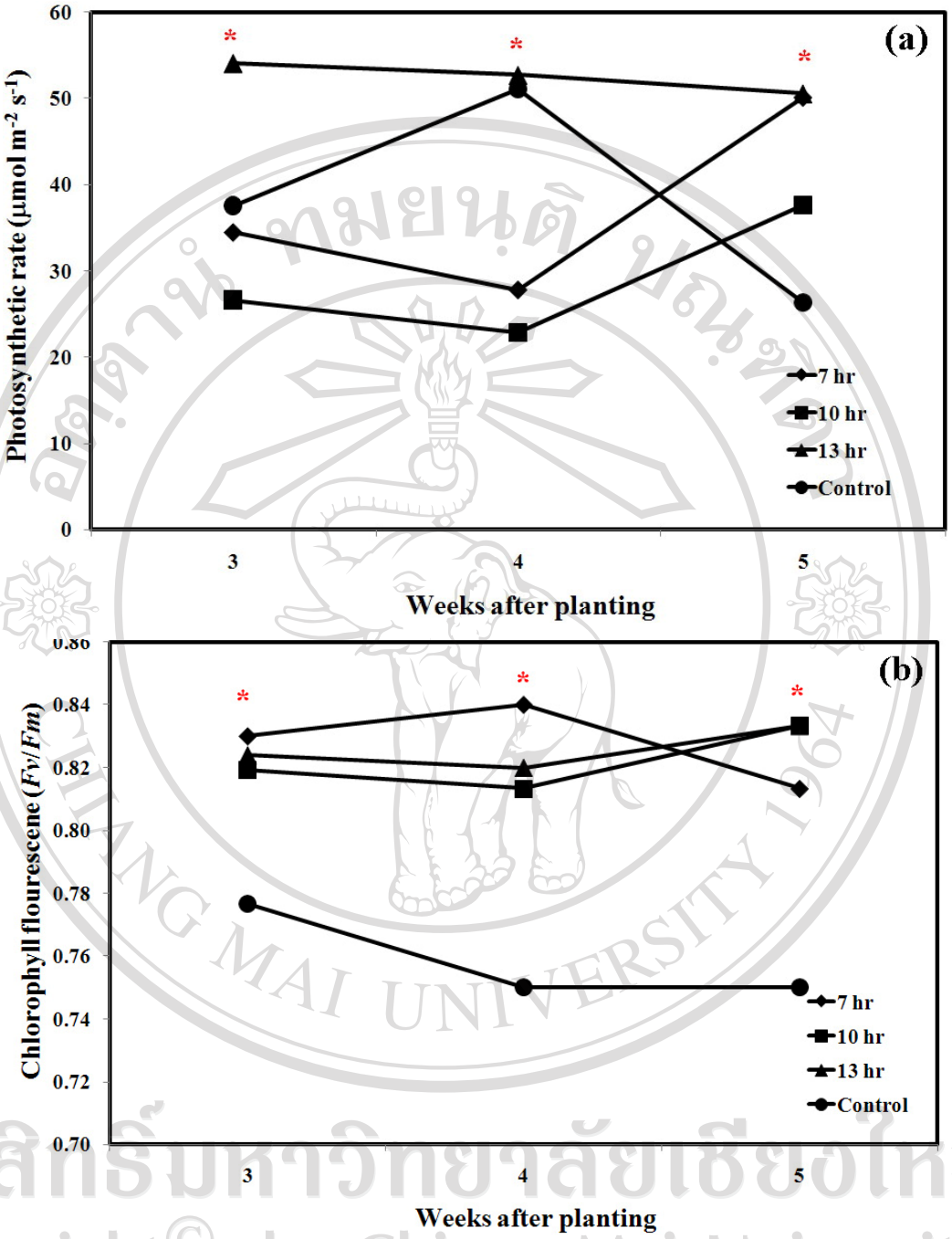


Figure 5.11 Photosynthetic rate (a) and Chlorophyll fluorescence (b) of plant growing under different day lengths compared with controlled treatment. Error bars denote the SE (n=10).

* indicated significant differences at *P<0.05*.

5.3.5 Total chlorophyll concentration

Table 5.3 shows the total chlorophyll, chlorophyll a and b in leaves of plants grown under different day length (7, 10 and 13 hrs) and natural condition (control) at 5 WAP. Total chlorophyll in leaves of plants under 7 hrs (0.67 g m^{-2}) was less than the others (10, 13 hrs and controlled with 0.74, 0.76 and 0.78 g m^{-2} respectively). Concentration of chlorophyll a and b tended to be the same as total chlorophyll (Table 5.3).

Table 5.3 Characteristics of chlorophyll in plants grown under different light sources at 5 WAP.

Day length (hrs)	Total chlorophyll (g m^{-2})	Chlorophyll a (g m^{-2})	Chlorophyll b (g m^{-2})
7	0.67±0.06b	0.52±0.04b	0.15±0.02b
10	0.74±0.03a	0.58±0.07ab	0.16±0.01ab
13	0.76±0.08a	0.60±0.09a	0.16±0.03ab
Control	0.78±0.04a	0.61±0.06a	0.17±0.08a

1/ Mean within the same column followed by different letters were significantly different between treatments at $P<0.05$

5.3.6 Biochemical content in rhizome and storage roots

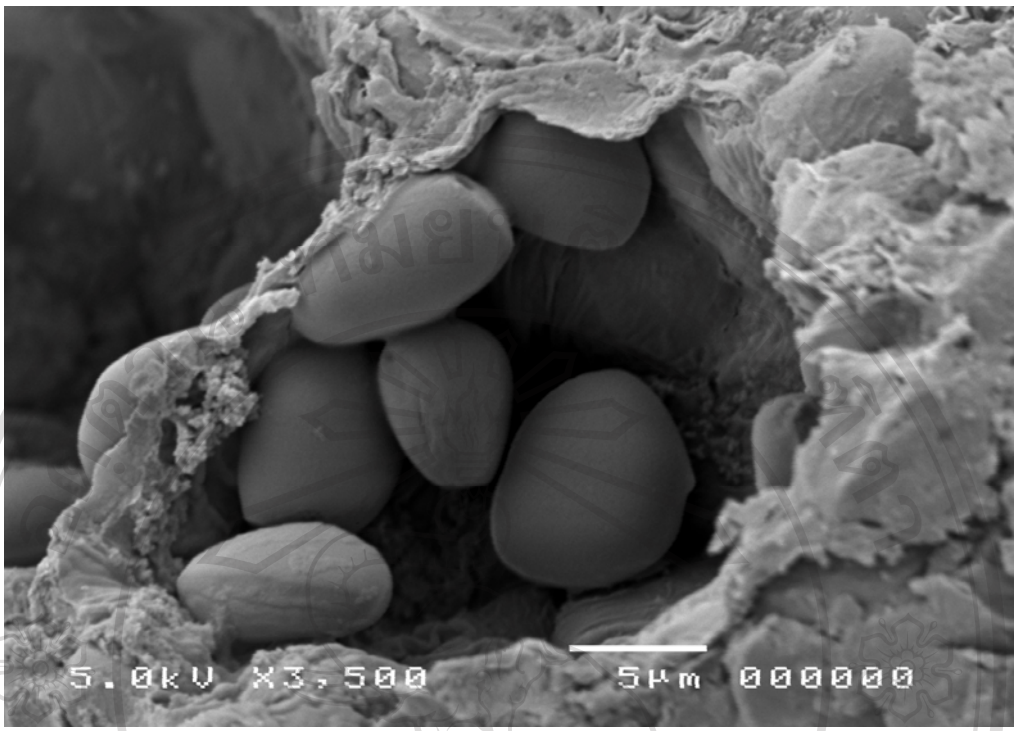
5.3.6.1 Total non structural carbohydrate (TNC) and Starch

Table 5.4 shows the data of TNC and starch. TNC in new rhizome of control was 283.52 mg-glucose/gDW and it was significantly different from that of 13 hrs (255.59 mg-glucose/gDW). Shorter day length (7 and 10 hrs) reduced TNC in rhizome and storage roots. Data of starch concentration were similar to TNC because these concentrations both in new rhizome and storage roots were reduced under shorter day length (7 and 10 hrs). Figure 5.12 shows the morphology of starch glandules in rhizome and storage roots grown under controlled treatment with oval elliptic and asymmetrical sphere shape. The glan size ranged from 3-10 μm of diameter.

Table 5.4 Total nonstructural carbohydrates (TNC), starch and total nitrogen in new rhizomes and storage roots of *C. alismatifolia* under different day length treatments at harvest.

Organ	Day length (hrs)	TNC (mg-glucose/gDW)	Starch (mg/g DW)
New rhizomes	7	226.80b	185.93c
	10	220.63b	261.44b
	13	255.59a	314.33a
	Control	283.52a	336.88a
Storage roots	7	170.34b	153.77c
	10	185.93b	202.47b
	13	236.84a	281.90b
	Control	268.65a	326.91a

[1/] Mean within the same column followed by different letters were significantly different between treatments at $P<0.05$



a

b

Figure 5.12 Morphology of starch in rhizomes (a) and storage roots (b) of *C. alismatifolia* under controlled treatment at harvest.

5.3.6.2 Total soluble sugar (TSS) and reducing sugar (RS)

Table 5.5 shows TSS and RS in new rhizome and storage roots. TSS concentration both in new rhizome and storage roots decreased when plants grew under shorter day length. RS of new rhizome under 7 and 10 hrs day length (14.54 and 17.10 mg-glucose/gDW, respectively) was lower than those of 13 hrs and under controlled (22.14 and 26.47 mg-glucose/gDW, respectively).

TSS in storage roots under control was 94.05 mg/g DW and it was higher than in other treatments. TSS in storage roots under different day length (7, 10 and 13 hrs) was increased when day length was longer (Table 5.5). Day length did not seem to affect RS concentration in storage roots, however, its concentration decreased on little bit at 13 hrs day length.

Table 5.5 Reducing sugars (RS) and total soluble sugars (TSS) in new rhizomes and storage roots of *C. alismatifolia* under different day length treatments at harvest.

Organ	Day length (hrs)	TSS (mg/g DW)	RS (mg-glucose/gDW)
New rhizomes	7	18.68c[1/]	14.54b
	10	22.02c	17.10b
	13	43.63b	22.14a
	Control	74.65a	26.47a
Storage roots	7	33.07c	64.06a
	10	40.42b	64.99a
	13	53.44b	57.48b
	Control	94.05a	66.96a

[1/] Mean within the same column followed by different letters were significantly different between treatments at P<0.05

5.3.6.3 Total amino acid concentration

Table 5.6 shows the concentrations of total amino acid in rhizome and storage roots under different day lengths compared with controlled ones. Total

amino acid in new rhizome under long day length (13 hrs) and control was higher than in short day length (7 and 10 hrs).

Table 5.6 Total amino acids in new rhizomes and storage roots of *C. alismatifolia* under different day length treatments at harvest.

Organ	Day length (hrs)	Total amino acid (mg-N/gDW)
New rhizomes	7	2.23c
	10	5.91c
	13	10.93b
	Control	59.34a
Storage roots	7	6.73c
	10	10.05b
	13	12.84b
	Control	15.70a

[1/] Mean within the same column followed by different letters were significantly different between treatments at $P<0.05$

5.3.6.4 Other free sugar (Fructose, Glucose and Sucrose)

Table 5.7 shows the concentrations of fructose, glucose and sucrose in rhizome and storage roots under different day length compare with controlled. Free sugar in new rhizome under long day length (13 hrs) and under control was higher than in short day length (7 and 10 hrs).

Free sugar in storage roots was significantly different among treatments. Fructose and glucose concentrations in storage roots under control (69.01 and 24.03 mg/g DW) were higher than other treatments. On the other hand, sucrose concentration under control was the lowest.

Table 5.7 Free sugars (Fructose, Glucose and Sucrose) in new rhizomes and storage roots of *C. alismatifolia* grown under different day light treatments at harvest.

Organ	Day length (hrs)	(mg/g DW)		
		Fructose	Glucose	Sucrose
New rhizomes	7	11.66c[1/]	5.98b	0.23b
	10	17.64c	3.35c	0.89b
	13	29.07b	9.93a	4.34a
	Control	59.26a	8.52a	6.25a
Storage roots	7	22.87c	9.85c	0.67c
	10	28.51c	9.14c	1.88b
	13	35.62b	14.33b	3.19a
	Control	69.01a	24.03a	0.05d

[1/] Mean within the same column followed by different letters were significantly different between treatments at $P<0.05$

5.3.6.5 Nutrient concentration

Table 5.8 displays the concentration of nutrient elements in new rhizome and storage roots grown under different day length condition. The concentration of nitrogen (N), potassium (K) and calcium (Ca) in new rhizomes under controlled treatment was higher than that of 10 and 13 hrs. However, the phosphorus (P) and magnesium (Mg) in new rhizome was not significantly different among the treatments (Table 5.8).

The concentrations of nutrient elements (N, P, K, Ca and Mg) in storage roots under natural condition were higher than in other treatments (Table 5.8). Day length did not affect concentrations of N, K and Mg in storage roots because these concentrations were not significantly difference between 7, 10 and 13 hrs. The concentration of P and Ca in storage roots under 13 hrs day length and was a little higher (10.41 and 11.67 mg/g DW) than in those of 7 and 10 hrs.

Table 5.8 Concentrations of nutrients in new rhizomes and storage roots under different day length treatments at harvest.

Day length (hrs)	Concentration in new rhizome (mg/ g DW)				
	Nitrogen [1/]	Phosphorus [ns/]	Potassium [1/]	Calcium [1/]	Magnesium [1/]
7	21.01±1.33a	6.98±0.32	166.07±8.22c	4.46±0.33a	3.17±0.12
10	21.53±8.25b	7.08±0.38	262.52±3.45b	3.56±1.50b	3.39±0.56
13	22.42±2.63b	7.38±0.54	344.46±9.47a	3.42±0.71b	2.37±0.39
Control	39.56±2.41a	6.83±0.63	350.30±5.21a	4.82±1.78a	2.88±0.95
Day length (hrs)	Concentration in storage root (mg/ g DW)				
	Nitrogen [1/]	Phosphorus [1/]	Potassium [1/]	Calcium [1/]	Magnesium [1/]
7	10.37±0.57b	8.49±0.13b	86.22 ±5.39b	1.47±0.36b	4.77 ±0.40b
10	9.52±1.35b	6.43±0.55c	81.85±9.18b	1.40±0.37b	3.61±0.27b
13	8.84±0.97b	10.41±0.77a	86.17±7.41b	2.95±0.31a	4.23±1.15b
Control	56.23±2.45a	11.67±0.13a	184.73±8.27a	2.44±0.76a	6.50±2.23a

[1/]: Mean within the same column followed by different letter were significantly different between treatment at $P<0.05$

5.3.7 Gene expression in rhizome under different day length

The RNA extraction from rhizome by Phenol/SDS method was able to yield RNA, however, with smeared background (Fig. 5.13a). The additional RNA cleanup was carried out by RNeasy© Kit, and bands corresponding to 18S and 28S rRNA were distinctly visible in all lanes, indicating high quality and low-degraded RNA (Fig. 5.13b). The total RNA of 7 and 13 hrs day length samples contained low amounts of contaminating proteins. The concentrations of RNA at 7 and 13 hrs day length were 1.62 and 1.58 μg/μl respectively The RNAs obtained following cleanup step by RNeasy© Kit were of good yield and quality. Differential gene expression in rhizome of *C. alismatifolia* was found by a total of three polymorphic bands (arrows; A_{1-3}, Fig. 5.13c).

Tables 5.9 shows the nucleotide sequences of 4 selected clones isolated from rhizome grown under 13 hrs day length. All generated sequences were entered into GenBank BLASTN to compare with all plant genes in public database. These results

indicat that the sequences of rhizome grown under 13 hrs day lengths were similar to other plant sequences as short sequences of about 17-20 bp were homologous to those of *Oryza sativa*, *Arabidopsis thaliana* and *Lotus japonicas* (Table 5.10).

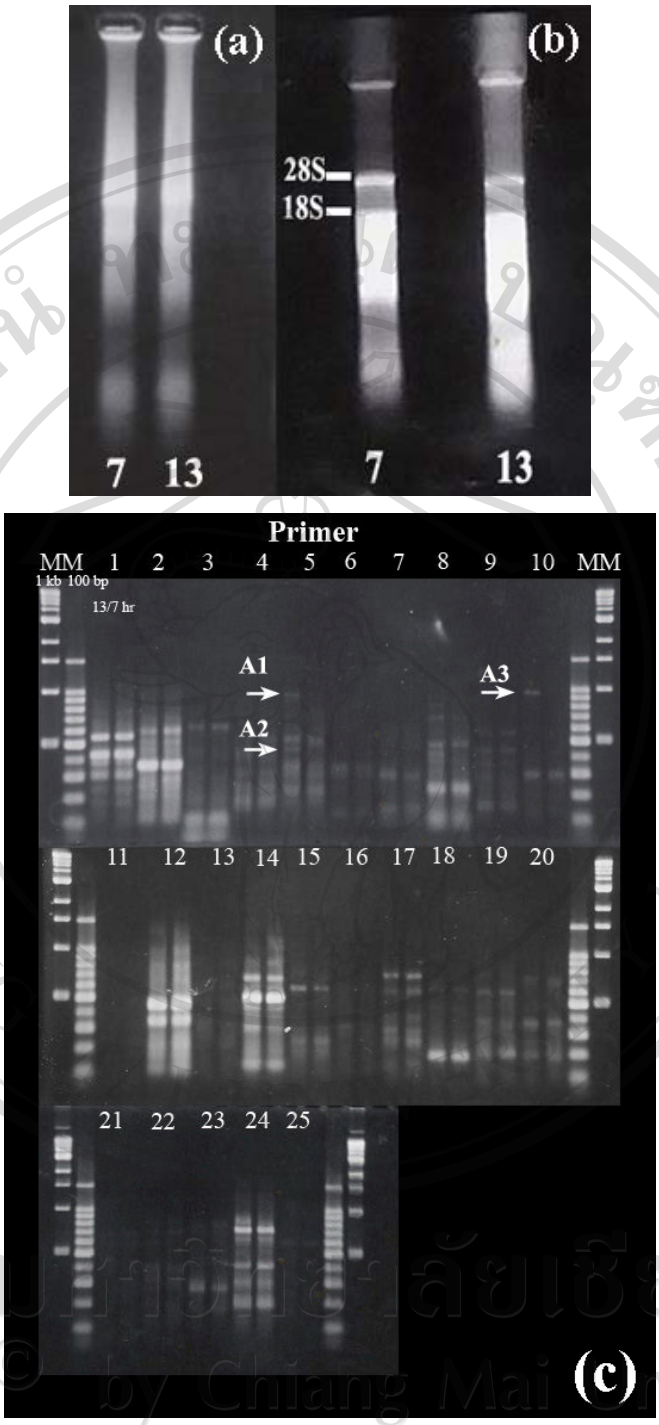


Figure 5.13 The total RNA of 7 and 13 hrs day lengths as extracted by Phenol/SDS method (a) and cleaned up by RNeasy© Kit (b). Agarose gel electrophoresis analysis of the nested PCR products rhizome of curcuma grown under different light sources (7 and 13 hrs) (c).

Table 5.9 The nucleotide sequences isolated from rhizome of *C. alismatifolia* grown under 13 hrs day length.

Sample of clones	Nucleotide sequences
No 2	GGAACCAATCTCACTTTCTTTAAATTTATTCCATTCCTTTCAACCTCA TTTATTTCAATGAGGTACTTCCAATTCCTAGAACTGTCTAGTCCATT ATCTTTTAATGCATGACCACCAACATCAAATCCTTCTACAATTGAGG ATCTACACAAAMCATTGCTGCTCACCTAACACTGTTTCTTATTCTAT ATCCCATCTCTCATTGAATCTCATATKCTCTAGGAAGaCACTTTAAG TGaGGWTgAAGTCACTTCAAtTAGgATACTGaTKTgATGAAGcAaATCA aGGaTTAATTKTcAMTtcAaaAgGMAGGATCATAATTACgCATtYCatgTG tAGTACTgTGTGTCATGATCCGGGCSCWGGGSGGAGCaTGWGTKgGG CCAATTScCTTgAKGKKGTTTAATTaTGScgTgTTTAAagGKGGNtTGGG AAaCCKGGSTGaGCGAKAKATATNACaGAgASAaAANMNaTATANWM TcABGtNBNSNSNVNMNaNANANAAAaAAAWaAAATTSaatTTNSTSaV RAATVSSSTTVSaSaS
No 5	GGAACCAATCCTAATGCTGCAACAGAACTGTTAGAAATCAAGGGA GTGTTGGATGCAATTGTGCAGCACCTGAGGAAACGTAAATTTTTGC TTGGCTTTTTATGTATTCCTTACTTTTAGGTTTGAGCGAATTTTTGTT ACCTCTTCTAATCTATAGATAGGATCATAAGACCCTTTTCACAATAG CTTAAGCTCTTAGTCAGACAGTTAAGTAAATTTATCTTGTACATCAT ACTAGGCTCATTTATGTTTCAATCCGGTCCATGCTCTTTTTTTTGGCC ATGTTCTTCATTCAACTGTCAAAAGGGTTTAATTACAGTAACCTGTG GTTGGTCTTTTCCGCCGGGGCTGGGGCATCCTGGGGTATATGCGCTT AGGGGCTGACGGTCATAGTTGGAACTGCGGAAGGCCCGTCCTCATT GATGATGGGTGAGACT
No 9	GGAACCAATCTAGCTTTGTTTCTAGTTACATTTCCTTGTTCATCAAG CTTATTCCTAAATACCCATTTGGTAGTGACAATTGTATTGTCATTTG GTCTAGGAACTAACTCCCACACATCACTCCTCTCAAATTGATTTAAC TCTTCTTGCATTGTTAAAATCCAATTTGAGTCATTTTAAAGCTTCAT CAATGTCTTTGGTTCAGTTGAGAATTAGTGCTACTTGAGTTTTTCAT TCTGAAGGGTGACCAGTTCTTACTCCTTGTTCATTATCCCAACAACT GATCTAATGGATGACTTGATGATGTTAATTTTCTATGGCCTAGGAGG ATATTAATTTTTAAAGGGCATATGGGGCTCCCCGGTTGTCATGATCC GGCCCCTGGAGGGCATCTAGGGTTATAGCTGTTAAGGGTGGACCGG TCATTAGCTAAATCTTCATGAAGGGGCCCTCCTCATGGTTTGATGGC TGGGTTGGGGGTGT
No 10	GTTTTTTTTTTTTTTTCATATGCTCTTCAATATATGCCCAACAAACAT GGATTAAATAATAAAATGCCTTTCTACCATTTGTAGGCCTGAAGAT CTACGTATACCATTCGAGCGTTTTTGGTCCAGTGAAGGATGTCTACC TTCCGAAGAACTACTATACAGGTACTTTGCCAACTGCAGTGAACTT ATTTTTCTCTCTCATTACAAGCATTAGGTCCTACAGTTGACTTATGG ACCATACTATTTCTTCTGGTGCCCTTTTTGGTAGGCAGGGTAGGGCA TTATATTAGGTATGACTGATAGGACTAGGGAGTATTCCATCCCTCCC GC

Table 5.10 List of differentially expressed fragments isolated from *C. alismatifolia* in comparison to other plant genes.

Sample of clones	Size (bp)	Best homology [1/]	ID number	E-value
No 2	19	*Arabidopsis thaliana* chromosome 3	NC_003074.4	0.18
	17	*Oryza sativa* (japonica cultivar-group)	NT_079865.2	0.78
	17	*Arabidopsis thaliana* chromosome 5	NC_003076.4	0.78
	18	*Arabidopsis thaliana* chromosome 4	NC_003075.3	0.78
	18	*Arabidopsis thaliana* chromosome 2	NC_003071.3	0.78
	18	*Arabidopsis thaliana* chromosome 1	NC_003070.5	0.78
No 5	18	*Oryza sativa* (japonica cultivar-group)	NT_107210.1	2.80
	18	*Oryza sativa* (japonica cultivar-group)	NT_107200.1	2.80
	18	*Oryza sativa* (japonica cultivar-group)	NT_107152.1	2.80
	18	*Oryza sativa* (japonica cultivar-group)	NT_107145.1	2.80
	18	*Oryza sativa* (japonica cultivar-group)	NT_107133.1	2.80
No 9	20	*Lotus japonicas* genomic DNA, chromosome 1	AP006429.1	0.20
	19	*Oryza sativa* (japonica cultivar-group)	NT_107206.1	0.79
	19	*Oryza sativa* (japonica cultivar-group)	NT_107179.1	0.79
	19	*Oryza sativa* (japonica cultivar-group)	NT_107175.1	0.79
	19	*Oryza sativa* (japonica cultivar-group)	NT_079879.2	0.79
No 10	20	*Oryza sativa* (japonica cultivar-group)	NT_107206.1	0.13
	20	*Oryza sativa* (japonica cultivar-group)	NT_107206.1	0.13
	20	CV02008B1H07.f1 CV02-normalized library	DV451947.1	0.13
	19	*Oryza sativa* (japonica cultivar-group)	NT_107216.1	0.53
	19	*Oryza sativa* (japonica cultivar-group)	NT_107177.1	0.53

[1/] Homology search was conducted with the NCBI BLAST program.

5.4 Discussion

The longer photoperiod (13 hrs) increased the height of the plant, as similar as in the report of Kuehny (2002). The intensity and duration of illumination requirement vary among plant species. Flowering plants have high light intensity requirements 6,000 to 10,000 lux, and most foliage plants need from 1,000 to 6,000 lux, flowering bulbs need 500 to 1,000 lux (Barkley, 2005). *C. alismatifolia* grown under 13 hrs of day length flowered at 6 WAP while floral abortion occurred in 7 and 10 hrs day length under low light intensity. The result indicated that day length did not affect floral initiation since floral bud were initiated in all treatments. However, short day inhibited the floral development and flowering under low light intensity. Ruamrungsri *et al*. (2005) found out that under low temperature during winter of Thailand, extended day length using night break treatment by supplying 2 hrs of an artificial light source could promote the flowering percentage of this plant. Day length did not affect the number of new rhizomes per plant and the number of storage roots at dormancy under low light intensity.

The rate of photosynthesis is the photosynthetic turnover per unit time which involved the process of CO_2 uptake, O_2 release and production of organic material (dry matter) (Lawlor and Lawlor, 1995). The photosynthetic rate and chlorophyll fluorescence were not significantly different. In chrsysanthemum, day length also gave the least effect on the photosynthesis rate when compared with temperature acclimation treatment *in vitro* (Adams and Fayyaz, 1979), although the photoperiod is important factor that affects photosynthetic rate as reported in eggplant seedlings (Kuehny *et al*.,2002) and soybean plants (Ohashi *et al*.,2006).

Total non structural carbohydrate (TNC) was determined as carbohydrates rsearve in plant witch starch is the most important reserve in higher plant (Wang *et al*., 2005). Total soluble sugars (TSS) comprised of various kinds of sugar such as monosaccharides and disaccharides in plant. Reducing sugars is one sugar group of TSS which contain a reaction carbonyl group, they are reddily oxidized to diverse products. Glucose, maltose, cellobiose and lactose are classified as reducing sugar while sucrose are classified as nonreducing sugars (Horton *et al*., 1996).

The content of TNC, starch, TSS and reducing sugar (RS) in new rhizome and storage roots of plants under short day length for 7 and 10 hrs treatment were lower

than in those under 13 hrs of day length which did not change the dry matter. Shanmugam and Muthuswamy (1974) reported that long day treatments increased carbohydrate levels concentrations in leaves of chrsysanthemum (short-day plant), which are similar to these in the rhizome and storage roots of *C. alismatifolia* (quantitative long day plant) in this experiment. The effect of day length on partitioning of assimilates between storage organs causes the interactions between light, darkness and circadian rhythms in component process of photosynthesis and sucrose biosynthesis. Phasing of the rhythms by light appears to be mediated by phytochrsome. However, the pattern of assimilate partitioning influences by day length is not consistent when plants grow under long term period. In *Poa pratensis* (long day plant), the stimulation of dry weight, plant height occurred without any change in the partitioning of assimilates in storage organs, on the other hand, barley plants grew under long day greater translocated assimilates to roots than plants grown at short day at the same irradiance (Thomas and Vince-Prue, 1997). In sugar beet and soy bean, short day decreased plant growth rate by decreasing photosynthate production but did not affect dry matter partitioning between roots and shoots (Rao *et al*., 1993) which was similar to *C. alismatifolia*.

Starch granules come in a wide variety of sizes ranging from 3 µm to over 100 µm. Granule shape also can be diverse. Granule shapes include symmetrical spheres, asymmetrical spheres, symmetrical disks and asymmetrical disks. Some granules exhibit their shape smoothly, while others are polyhedrons with a faceted surface (Hegenbart, 1996). Starch granule in *C. alismatifolia* is predominat in ovule, elliptic shape and asymmetrical sphere with the ranges of 3-10 µm diameters. The shape is similar to granule of starch in *Fritillaria* species but it is different in range of size (Wang *et al*., 2005). Granule size is contributing factor in how rapidly a starch gelatinizes and its gelatinization temperature (Hegenbart, 1996).

The influence of day length on mineral elements was different among nutrients. The N concentrations in new rhizomes and storage roots were not significantly affected by photoperiod. Ohtake *et al.* (2006) reported that N concentration both in the rhizomes and in the storage roots were increased by high level of N supply to curcuma plant increased. The P, Ca and Mg in new rhizomes, K and Mg in storage roots were not significantly different among day length treatments. The short day

length (7 hrs) decreased the concentrations of K in new rhizomes. The concentrations of P and Ca in storage roots decreased in the short day plants. In chrsysanthemum, short day also decreased N, K, Ca and Mg contents in leave (Shanmugam and Muthuswamy, 1974).

This red part has no link to your result. I don't think it is suitable to be pointed out here. Because phytochrome is one of photoreceptors which involve in the response of day length. Phytochrome action can ultimately lead to major stitches in gene expression. The changes in the chloroplast enzymes in response to phytochrome action are proportionally greater than those of the cytoplasmic enzymes, and that where related to growth parameters, including DNA content, the increase in activity of the cytoplasmic enzymes might simply be a function of increased cell division (Tobin and Silverthorne, 1985). This experiment revealed that there were three polymorphic bands differentially expressed when plants were grown under different day lengths, suggesting that there was the transcriptional regulation of genes in response to day length during rhizome formation. The isolated clones, however, were of short sequences and the homology search revealed matches to the sequences whose functions were not identified. This technique benefits gene expression analysis in relation to plant development which can be studied in individual samples in relation to the morphological changes as during rhizome formation. Photoperiod has been know to control several responses throughout the plant life cycle, like germination, flowering, tuber or rhizome formation, onset of bud dormancy, leaf abscission, and cambium activity (Martinez-Garcia, 2002). Thereby the isolated clones from treated rhizome under this study may somehow reflect the effect of photoperiod on gene expression.

5.5 Conclusion

It was concluded that short day (7 hrs) decreased the number of shoots and rhizomes per plant and number of storage roots per rhizome under sufficient light intensity in this experiment. Under low light intensity all the plants exhibited succulent growth, and produced only one shoot with one new rhizome. Short day treatment also decreased food reserve i.e. reducing sugar and K in new rhizomes and total nonstructural carbohydrate, reducing sugar, P and Ca in storage roots. Therefore,

day length is required for the rhizome production of *C. alismatifolia* as well as cut flower production. The result of gene expression showed that three polymorphic bands were differentially expressed when plants were grown under different day length conditions. Sequencing results indicated that the selected clones were not identical to any gene with known function in the NCBI database.