Chapter 6

Effect of red light on rhizome formation of *Curcuma alismatifolia*

6.1 Introduction

Manipulation of plant material in order to satisfy dormancy requires investigation on the individual plant requirements. It appears that controlling growth, development and flowering in geophytic plants depends on reserve accumulation, mobilization and redistribution (Phongpreecha, 1997). *C. alismatifolia* is herbaceous perennial with short fleshy rhizome and storage roots or tuberous roots. (Burch *et al*., 1987). The rhizome is a major source of water and carbohydrates. (Wannagrairot, 1997). *C. alismatifolia* has developed swollen roots to store water and reserve food for plant growth (Phongpreecha, 1997). Therefore, the knowledge of factors affecting these mechanisms would be useful for the development of cultural techniques that control shoot emergence, vegetative growth and flowering in order to guarantee a good quality of storage organs. Among environmental condition, light is one of the limiting factors and affects growth and morphogenetic mechanism of plants. Light quality shows an important role in morphogenesis and photosynthesis (Kim *et al*., 2003). After importing curcuma rhizomes from Thailand, growers in foreign countries usually produced curcuma plants in glass-houses using supplemental artificial light. Not only curcuma production in foreign countries, off-season production of *C. alismatifolia* in Thailand also used artificial light sources for the night-break technique to improve the quality of flowers and rhizome. Fluorescent light source as cool day light provide limited fluence rate and having little in far-red region (Hart, 1988). Red light is important for the development of the photosynthetic apparatus of plants and may increase starch accumulation in several plant species by inhibiting the translocation of photosynthates out of the leaves (Saebo *et al*., 1995). Armstrong (2007) revealed that use of red-colored netting over sweet paper can increase yield by 15-20%. The net reduced bule and green light and increase red and far-red.

Red-colored net may be useful for *C. alismatifolia*. However, little information is available on how red light influences the growth, photosynthesis and food reserves of this plant. This research, therefore, was aimed to understand the effect of red light on growth, development and nutrient assimilation of this plant for applying factor to improve quality of flowers and rhizome.

6.2 Materials and methods

6.2.1 Plant materials

The experiment was carried out using rhizomes of *Curcuma alismatifolia* cv. Chiang Mai Pink with a diameter of 2.5 cm with 4-5 storage roots grown in plastic bags (containing sand: rice husk: rice husk charcoal at the ratio of 1:1:1; Fig. 6.1a). After shoot and root emerged (about two weeks after planting :WAP), The plant were transferred to growth chambers. There were three different light sources i.e, 1) red light fluorescent lamps (Philips TLD 36W/15; 632 – 660 nm wavelength, Fig. 6.1b), 2) cool daylight source (Philips TLD 36W/865; 405-812 nm wavelength, Fig. 6.1c) and 3) the natural light source (control) (Fig. 6.1d). The condition in the growth room was set up at 27±2^{o}C, 70-80% RH and 60 µmol $m^{-2}s^{-1}$ PAR of light intensity. Each plant was supplied with 100 ml of nutrient solution comprised of 200 mg N, 50 mg P, 200 mg K, 65 mg Ca, 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 at three times a week. The experiments were conducted during the period of May 2004 to December 2004 at Lampang Agricultural Research and Training Center, Rajamangala University of Technology Lanna, Thailand.

a

b

c

d

Figure 6.1 Plant materials (a) grown under red light (b) cool day light (c) and natural light (d).

6.2.2 Data collection

6.2.2.1 Change of vegetative growth and physiological aspects

Ten plants of curcuma (replications) were sampled in order to collect data. The collected data were vegetative growth and the physiological change, as affected by different light sources, and were studied through the following parameters: photosynthetic efficiency, chlorophyll fluorescence, chlorophyll content, similar to chapter 5.

6.2.2.2 Gene expression in the rhizome formation by DD RT-PCR

Two rhizomes each from ten plants per treatment was sampled for gene expression analysis. The method was determined at Niigata University, Japan using the DD RT-PCR method as described in chapter 5.

6.2.2.3 Changes in biochemical contents in rhizome and storage roots

Similar to the determination of biochemical contents in rhizome and storage root described in chapter 5.

6.3 Results

6.3.1 Plant growth and development

Fig. 6.2 shows the growth and development of *C. alismatifloria* grown under different light sources. The plant height of cool daylight and red light sources was higher than in the controlled condition (Fig. 6.2a). Diameters of pseudo-stem of all treatments were not different at 1 to 7 WAP. After 8 WAP, the diameter of the pseudo-stems of the plants under red light conditions were significantly lower than under other treatments (Fig. 6.2b). The number of leaves per plant grown under controlled treatment was higher than under all treatments after 3 WAP (Fig. 6.2c).

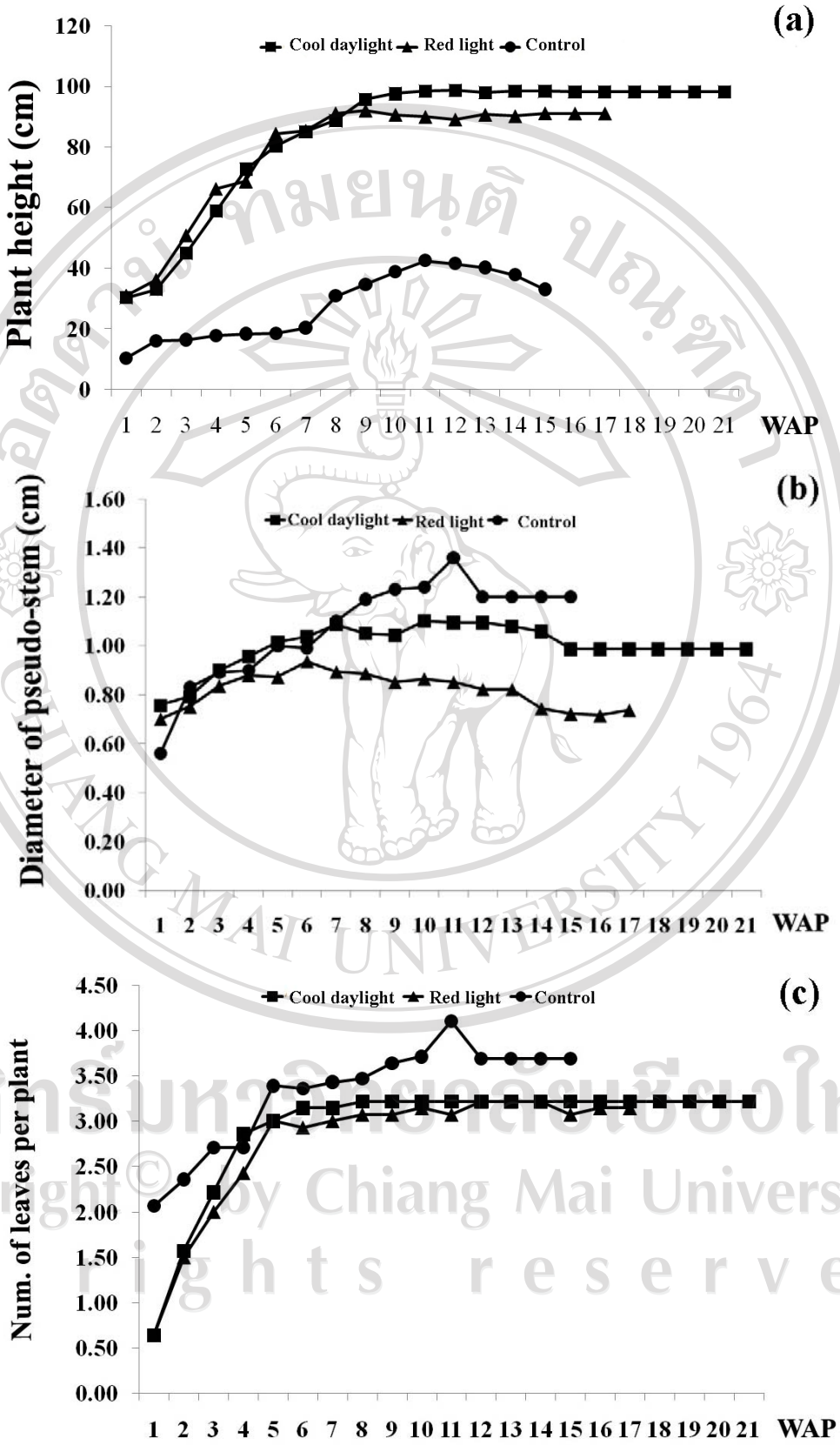


Figure 6.2 Growth characteristics of plants grown in different light sources.

Height of plants at 11 WAP grown under red light condition (92.30 cm) was not significantly different compared with cool day light (97.70 cm) and plants under both conditions were higher than with natural light (42.40 cm) (Table 6.1). The cause of abnormal elongation of plants under red and cool day light was due to low light intensity (60 µmol $m^{-2}s^{-1}$ PAR) in these conditions compared with the controlled in T3 (9,000 µmol $m^{-2}s^{-1}$ PAR), rather than the affect of light quality.

Moreover, the pseudo-stem diameter was smaller. the number of plants per clusters and the number of leaves per plant were lower than in natural light treatment (Table 6.1).

C. alismatifolia grown under red light and cool day light conditions began to flower early at 4 weeks after planting (4 WAP) (Fig. 6.3). Plants under red light reached the dormancy stage at 19 WAP, which was earlier than plants grown under cool day light and natural light (23 and 25 WAP, respectively).

Table 6.1 Growth of *C. alismatifolia* grown under different light sources at 11 WAP.

Light sources	Height (cm)	Diameter of pseudo-stem (cm)	Num. of plant per cluster	Num. of leaves per plant	Leave area (cm^2)
Cool day light	97.70[a1/]	1.11[b]	1.90[b]	3.30[b]	116[b]
Red light	92.30[a]	0.80[c]	1.40[b]	3.30[b]	93[b]
Control	42.40[b]	1.36[a]	2.90[a]	4.10[a]	162[a]

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

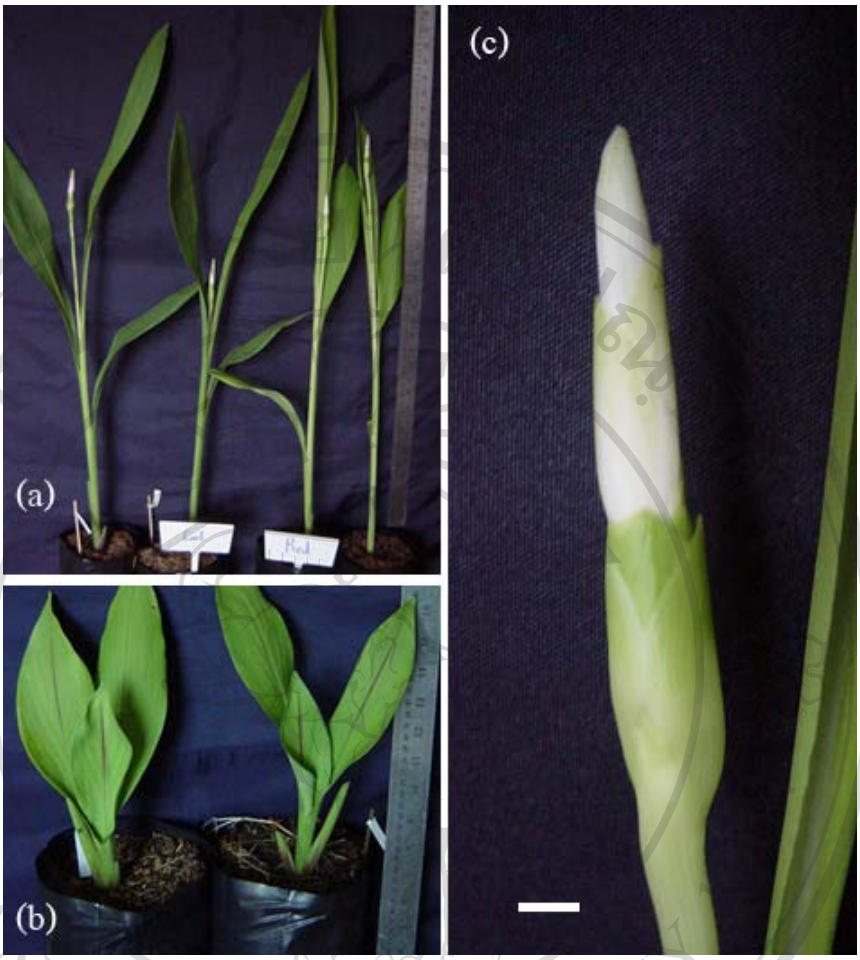


Figure 6.3 Flowering of plants grown under red light [(a) right] and cool day light [(a) left] conditions at 4 WAP. Plants grown under controlled treatment did not flower at 4 WAP (b). Flowers of plants grown under red light at 4 WAP (c). [bar = 1 cm]

Under red light treatment, the number of new rhizomes per cluster was 1.00 rhizome and it was not significantly different from those under cool day light (2.00 rhizomes per cluster). Under controlled treatment, the new rhizome was 3.67 rhizomes per cluster (Table 6.2). The diameter of new rhizome under red light condition (0.92 cm) was significantly lower than under other treatments. The fresh and dry weights of new rhizome under red light were lower than from plants under cool day light and controlled treatments (Table 6.2).

The number of storage roots per rhizome under red light condition and cool day light was significantly different from those under control treatment (1.67, 3.33 and

7.67 storage roots per rhizome, respectively). Diameter of storage root under red light was smaller than from the others (Table 6.2). The fresh and dry weight of storage roots under red light condition (13.73 and 0.91 g) was significantly lower than from plants under cool day light (15.92 and 2.07 g) and controlled treatment (17.55 and 2.20 g).

Table 6.2 Effect of different light sources on yield and qualities of new rhizomes and storage roots of *C. alismatifolia* at harvest.

New Rhizome				
Light sources	No. of new rhizomes per cluster	Diameter of new rhizome (cm)	Weight of new rhizome (g)	
			Fresh	Dry
Cool day light	2.00[b1/]	1.94[a]	5.43[b]	1.32[a]
Red light	1.00[b]	0.92[b]	3.23[b]	0.66[b]
Control	3.67[a]	2.30[a]	6.48[a]	1.66[a]
Storage roots				
Light sources	No. of storage roots per rhizome	Diameter of storage roots (cm)	Weight of storage roots (g)	
			Fresh	Dry
Cool day light	3.33[b1/]	1.30[a]	15.92[b]	2.07[b]
Red light	1.67[b]	0.90[b]	13.73[c]	0.91[c]
Control	7.67[a]	1.60[a]	17.55[a]	2.20[a]

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

6.3.2 Photosynthetic rate and Chlorophyll fluorescent

6.3.2.1 Photosynthetic rate

Photosynthetic rate of plant grown under controlled treatment was higher than under other treatments but it was not significantly different between cool daylight and red light (Fig. 6.4).

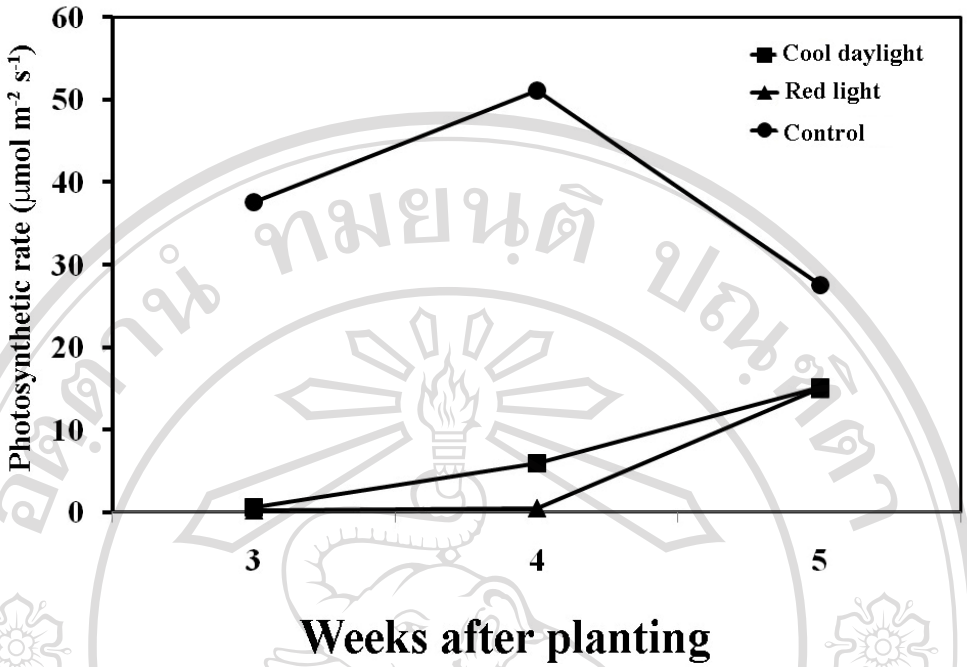


Figure 6.4 Photosynthetic rates of *C. alismatifolia* grown in different light sources.

The photosynthetic rate was measured by measuring the volume of carbondioxide with LCA-4 instrument at 10.00 am at 5 WAP. Photosynthetic rate of plants under red light was 14.95 μmol $m^{-2}s^{-1}$ and it was not significantly different from plant under cool day light (15.05 μmol $m^{-2}s^{-1}$), indicating that different wave lengths with the same light intensity between cool day light and red light did not affect photosynthetic rate. On the other hand, the photosynthetic rate of plants grown under controlled treatments was 27.56 μmol $m^{-2}s^{-1}$ which was significantly higher than under other treatments (Table 6.3), due to higher light intensity and leave area (Table 6.1).

Table 6.3 Photosynthetic rates of plants grown under different light sources at 5 WAP.

Light sources	Photosynthetic rate (μmol $m^{-2}s^{-1}$)
Cool day light	15.05[b1/]
Red light	14.95[b]
Control	27.56[a]

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

6.3.2.2 Chlorophyll fluorescence

Figure 6.5 shows the chlorophyll fluorescence of plants grown under different light sources; the data of the cool daylight and red light were higher than under controlled treatment. The chlorophyll fluorescence of plants grown under controlled treatments gradually decreased during 5 WAP.

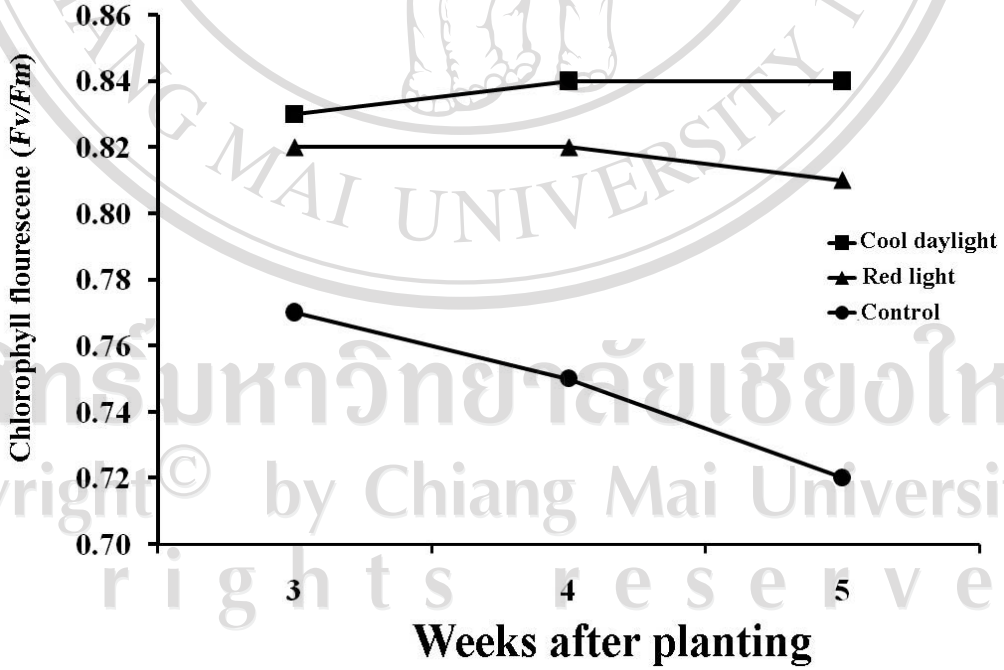


Figure 6.5 Chlorophyll fluorescence of *C. alismatifolia* grown under different light sources.

The chlorophyll fluorescence of *C. alismatifolia* leaves grown under treatment conditions at 5 WAP shown in Table 6.4. The *Fv/Fm* ratio indicated the effect of outside factors to chlorophyll efficiency and stress of plant (Flagella *et al*., 1994). Chlorophyll fluorescence values of *C. alismatifolia* grown under red light was 0.81 and it was not significantly different from plants grown under cool day light (0.84). However, values of both conditions were higher than from plants grown under natural light (0.72) (Table 6.4). Although, the total chlorophyll, chlorophyll A and chlorophyll B of plants grown under red light were lower than under other treatments (Table 6.4).

Table 6.4 Characteristics of chlorophyll in plants grown under different light sources at 5 WAP.

Light sources	Chlorophyll fluorescence (*Fv/Fm*)	Total chlorophyll	Chlorophyll a	Chlorophyll b
Cool day light	0.84[a1/]	0.88[a]	0.69[a]	0.19[a]
Red light	0.81[a]	0.63[c]	0.49[c]	0.14[c]
Control	0.72[b]	0.78[a]	0.61[a]	0.17[b]

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

6.3.3 Biochemical content in rhizome and storage root

6.3.3.1 Total nonstructural carbohydrate (TNC)

Figure 6.6 shows the concentration of total nonstructural carbohydrate in rhizome and storage roots of *C. alismatifolia* grown under different light sources. The TNC of rhizome and storage roots was slowly increasing from 3 to 13 WAP and actively increasing thereafter until 21 WAP. Plant grown under controlled treatment had higher concentration of TNC than under other treatments in both organs (Fig. 6.5).

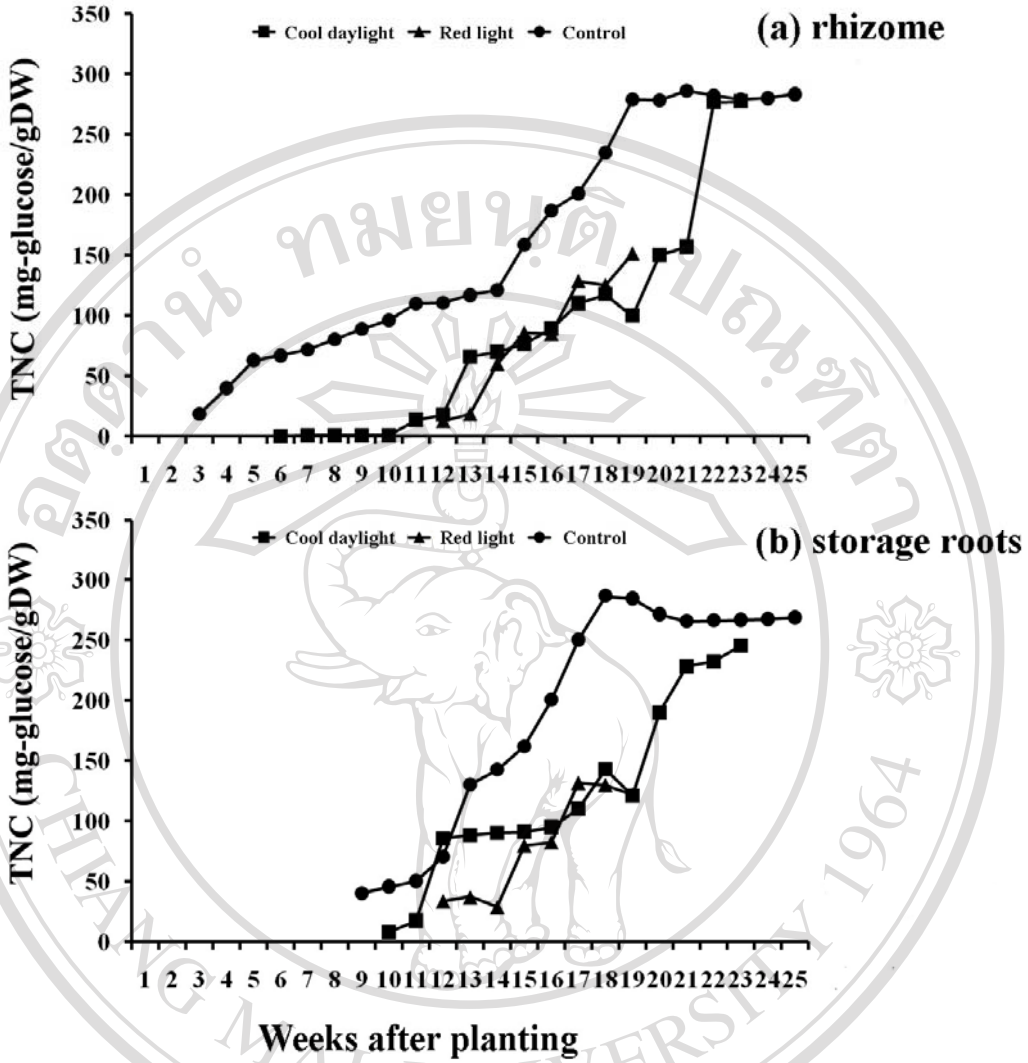


Figure 6.6 The total non-structural carbohydrates (TNC) of rhizome (a) and storage roots (b) grown under different light sources.

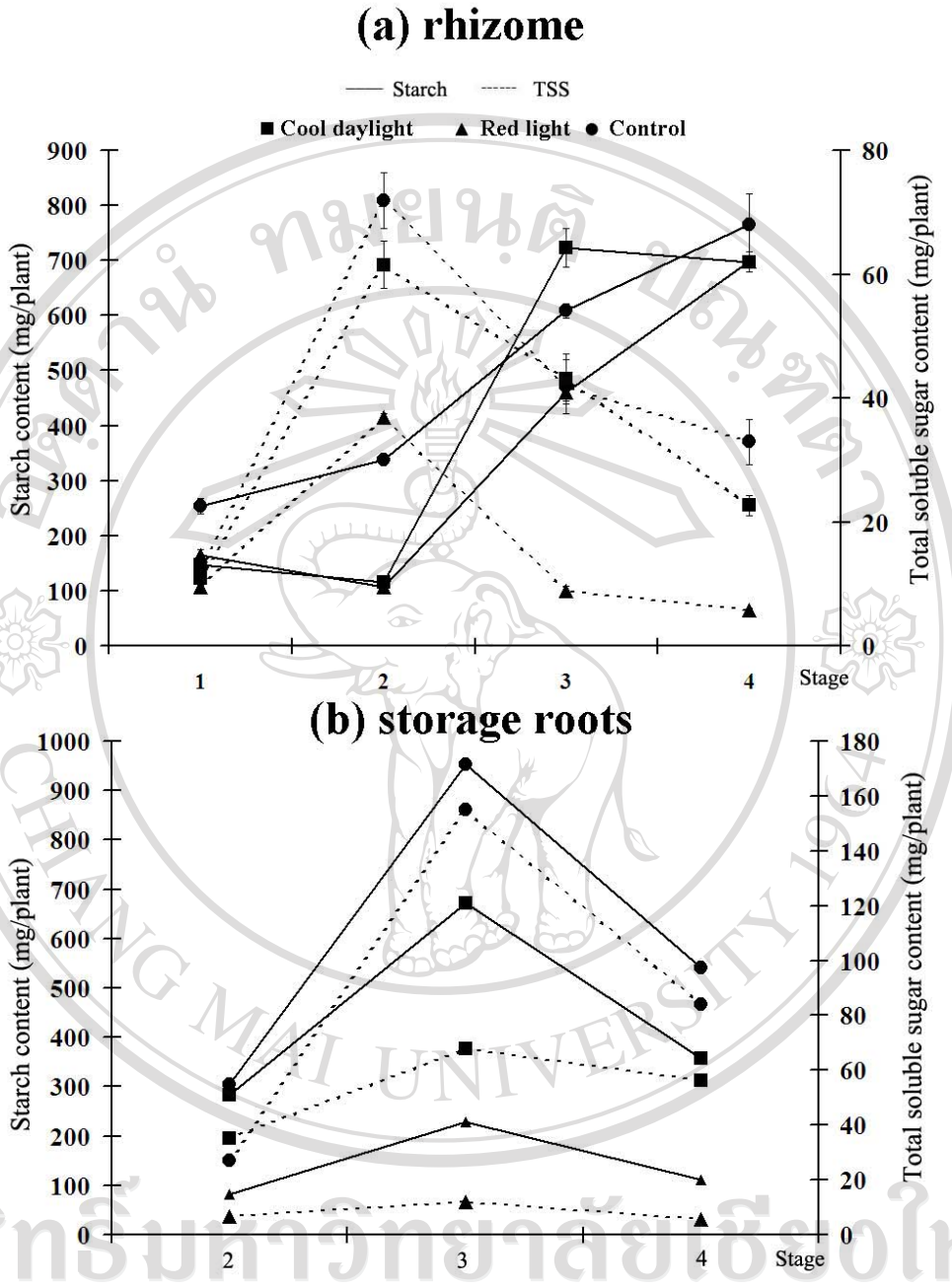
Total non-structural carbohydrate at harvest of new rhizome and storage roots under red light condition were 151.35 and 122.05 mg/gDW, respectively and they were less than under other conditions (Table 6.5). The starch concentration in new rhizome and storage roots under red light (93.68 and 162.94 mg/gDW, respectively) were significantly lower than in cool day light (183.43 and 222.45 mg/gDW, respectively) and under controlled treatment (336.88 and 326.91 mg/gDW, respectively). Because the photosynthetic rate was not significantly different among treatments (Table 6.5).

6.3.3.2 Starch concentration

The starch contents in new rhizome of all treatments continuously increased from stage 1 to stage 3 (compact spike senescence). Under natural light, its contents were 254.12, 338.57, 608.59 and 765.25 mg per plant at stage 1, 2, 3 and 4, respectively (Fig. 6.7a). Red light treatment decreased starch contents in new rhizome compared with treatment in natural light at stage 1, 2 and 3. It was interesting that in storage roots, the contents continuously increased from stage 2 to stage 3 and it reached maximum at stage 3 when compact spike were senescence (Fig. 6.7b). Then the starch contents in all treatments were rapidly decreased from stage 3 to stage 4 (rhizome harvest). The content of starch in storage roots of plants grown under red light was 107.21, 460.23 and 697.82 mg per plant at stage 2, 3 and 4 and was significantly lower than under other treatments. It was not significantly different from cool day light and natural conditions at harvest (stage 4).

6.3.3.3 Total soluble sugar (TSS) concentration

Total soluble sugar contents in new rhizome (Fig. 6.7a) under all treatments reached maximum levels at stage 2 and gradually decreased until rhizome harvest at stage 4. Its contents in plants grown under red light were 36.84, 8.89 and 5.76 mg per plant at stage 2, 3 and 4, respectively, and they were significantly lower than under other treatments. On the other hand, their contents in storage roots were at maximum at stage 3 and gradually decreased at stage 4. The content of total soluble sugar in storage roots of plants under red light treatment was lower than under those of cool day light and natural condition treatments (Fig. 6.7b). It was interesting to note that total soluble sugar contents in storage roots tended to be higher than those in new rhizomes. TSS of new rhizome and storage roots under red light was not significantly different with cool day light condition. But, those concentrations under both conditions were lower than in natural light conditions (Table 6.5).



Stages of growth : 1 = Leaves fully expand, 2 = The first floret opened, 3 = Compact spike senescene, 4 = Rhizome harvest

Figure 6.7 Carbohydrate content in the new rhizome: a) and storage roots ; b) of *C. alismatifolia* on different light sources.

Table 6.5 Total non-structural carbohydrates (TNC) and starch in new rhizome and storage roots of *C. alismatifolia* under different light source treatments at harvest.

Organ	Light sources	TNC (mg-glucose/gDW)	Starch (mg/g DW)
New rhizomes	Cool day light	277.35[a1/]	183.43[b]
	Red light	151.35[b]	93.68[c]
	Control	283.52[a]	336.88[a]
Storage roots	Cool day light	245.57[b]	222.45[b]
	Red light	122.05[c]	162.94[c]
	Control	268.65[a]	326.91[a]

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

6.3.3.4 Reducing sugar (RS) concentration

Figure 6.8 shows the concentration of reducing sugar in rhizome and storage roots grown under different light sources. The RS of rhizome grown under red light is lower than under other conditions (Fig. 6.8a). In storage roots, RS concentration of plants grown under controlled treatment actively increased and reached a maximum at 18 WAP and become constant thereafter (Fig. 6.8b). Pattern of RS concentration in cool day light treatment was the same as under controlled treatment, and reached a maximum at 20 WAP.

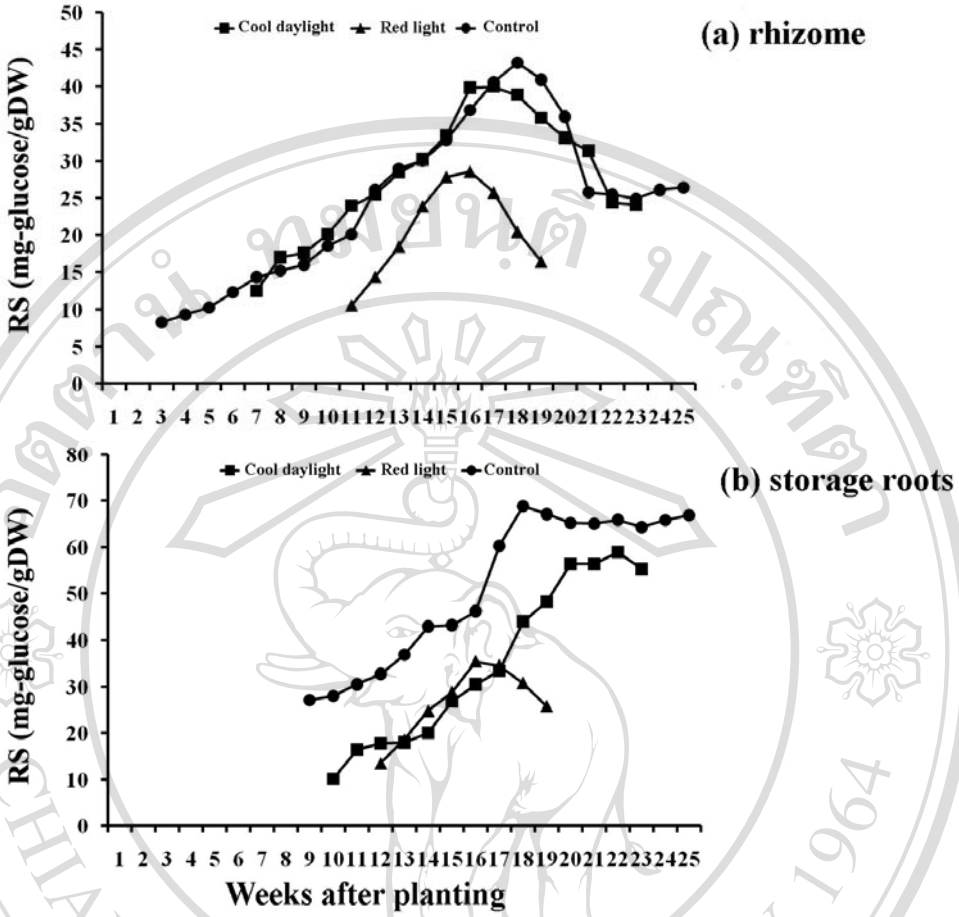


Figure 6.8 The reducing sugars in rhizomes (a) and storage roots (b) grown under different light sources.

In table 6.6, the reducing sugar (RS) concentration in new rhizome and storage roots of plants under red light condition (16.44 and 25.81 mg-glucose/gDW, respectively) were significantly lower than in those of cool day light (24.11 and 53.36 mg-glucose/gDW, respectively) and in those of natural light (26.47 and 66.96 mg-glucose/gDW, respectively).

Table 6.6 Reducing sugars (RS) and total soluble sugars (TSS) in new rhizomes and storage roots of *C. alismatifolia* under different light source treatments at harvest.

Organ	Light sources	TSS (mg/g DW)	RS (mg-glucose/gDW)
New rhizomes	Cool day light	44.04[b]	24.11[a1/]
	Red light	43.34[b]	16.44[b]
	Natural light	74.65[a]	26.47[a]
Storage roots	Cool day light	51.77[b]	53.36[b]
	Red light	47.37[b]	25.81[c]
	Natural light	94.05[a]	66.96[a]

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

6.3.3.5 Free sugar (Fructose, Glucose and Sucrose)

Figure 6.9 shows the changes of each kind of free sugar contents in the new rhizome (Fig 6.9a) and storage roots (Fig. 6.9b) at different stages of growth affected by different light sources. After planting, the content of fructose, glucose and sucrose in new rhizome and storage roots increased until anthesis (stage 2) and decreased thereafter. Sucrose in new rhizome was detected after the anthesis stage, and it was the highest content of free sugar in the new rhizome and storage roots. Fructose, glucose and sucrose contents in new rhizomes of plants grown under red light were 6.65, 8.32 and 8.86 mg per plant respectively and they were significantly lower than in those of cool day light and controlled treatments. In storage roots, plants accumulated maximum contents of fructose (4.44 mg per plant), glucose (6.73 mg per plant) and sucrose (3.43 mg per plant) at stage 3 (compact spike senescence) under control treatment, different from those of new rhizomes which was the highest at stage 2. Higher contents of these free sugars were found in plants grown under control treatment. However, most of them decreased at harvest stage.

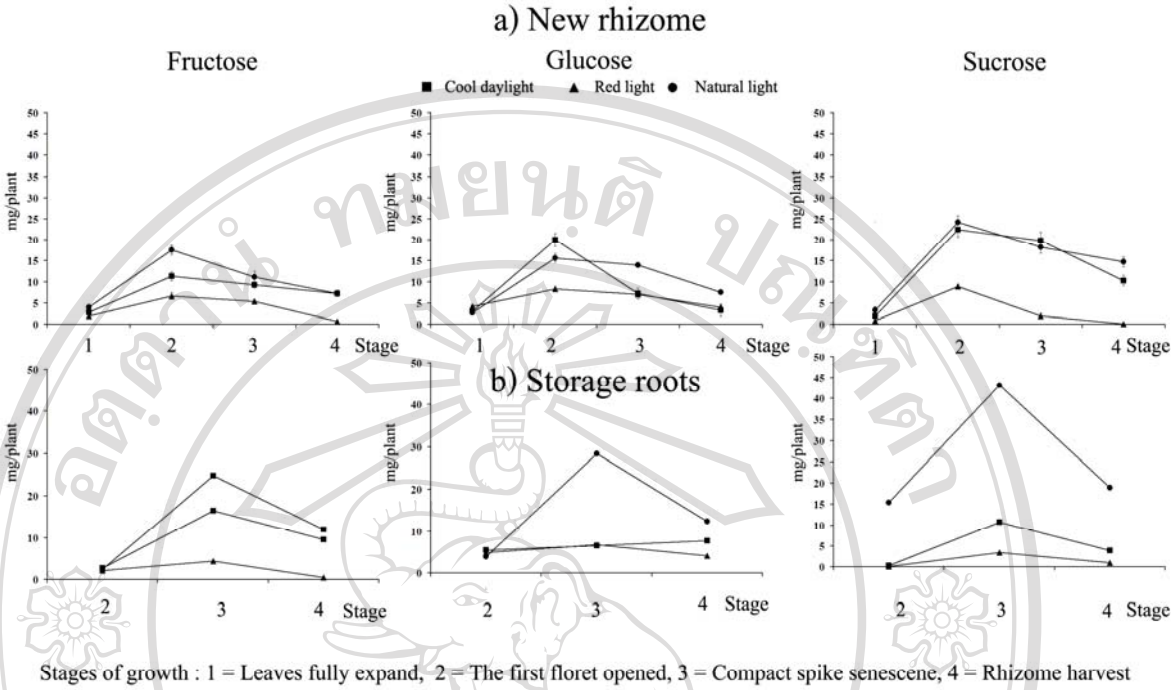


Figure 6.9 Fructose glucose and sucrose contents in the new rhizomes (a) and storage roots (b) of *C. alismatifolia* on different light source.

6.3.3.6 Total nitrogen concentration

Total nitrogen of new rhizome and storage roots under red light condition was lower than under other treatments (Table 6.7). The total nitrogen of new rhizome under red light, cool day light and natural light was 25.84, 36.70 and 59.34 mg-N/gDW, respectively.

Table 6.7 Total nitrogen in new rhizomes and storage roots of *C. alismatifolia* under different light source treatments at harvest.

Organ	Light sources	Total N (mg-N/gDW)
New rhizomes	Cool day light	36.70[b]
	Red light	25.84[c]
	Control	59.34[a]
Storage roots	Cool day light	12.63[b]
	Red light	10.17[c]
	Control	15.70[a]

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

6.3.4 Gene expression during rhizome formation by DD RT-PCR

The total RNA was extracted from rhizome by the method as used in chapter 5. Figure 6.10a shows the yield of total RNA with smeared background. 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. 6.10b). The total RNA of red light and natural light samples had low amounts of contaminating proteins. The concentrations of total RNA of red light and natural light became lower (1.61 and 1.61 μg/μl, respectively) following cleanup step by RNeasy© Kit, however, yield and quality of RNA extracted from rhizome were well obtained. Differential gene expression in rhizome of *C. alismatifolia* was found in a total of four polymorphic bands (arrows; B_{1-4}, Fig. 6.10c).

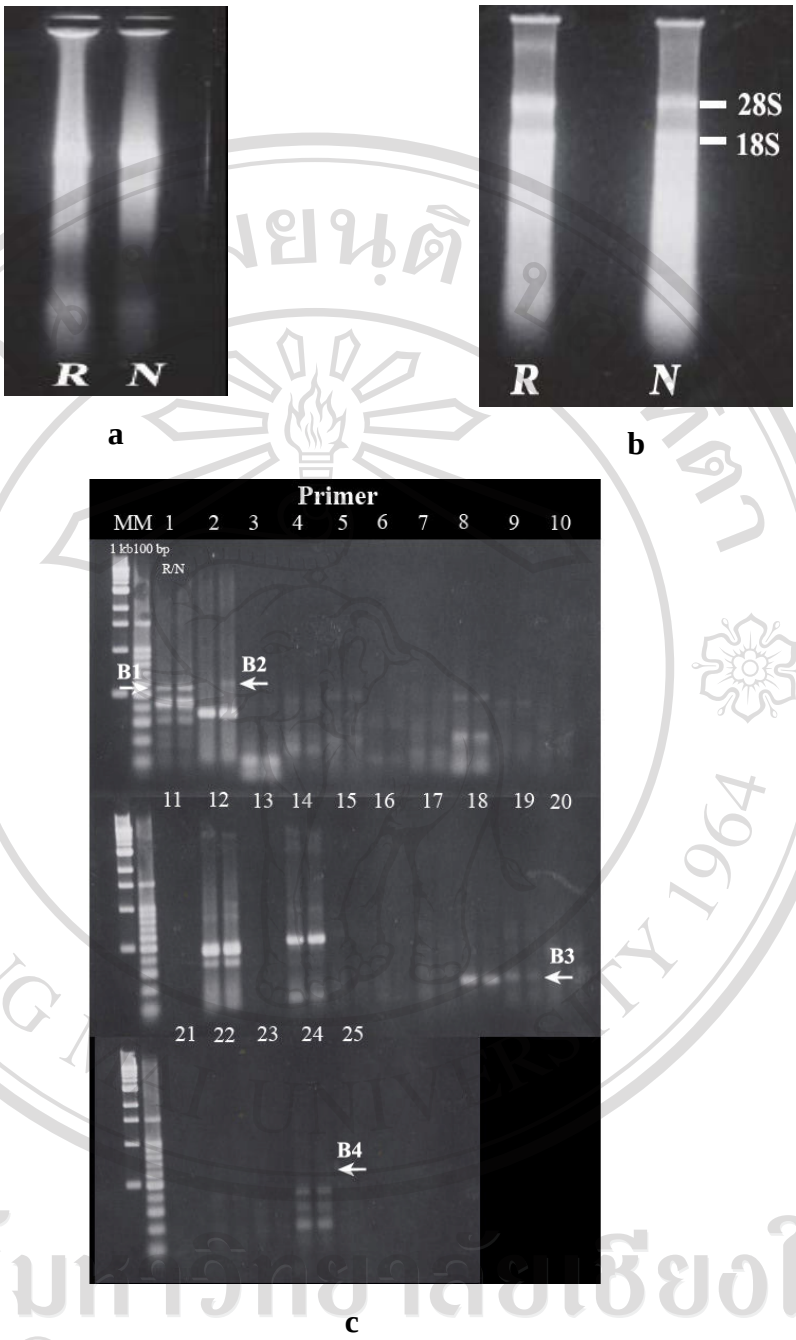


Figure 6.10 The total RNA of red light and natural light as extracted by Phenol/SDS method (a) and cleaned up by RNeasy© Kit (b). Agarose gel electrophoresis analysis of the nested PCR products rhizome of curcuma grown under different light sources (Red light; R and Natural light; N)(c).

6.4 Discussion

Plant can use all colors of light in photosynthesis although green and yellow light are used less efficiently, often around 60% of red and blue light. Plant grown with on red light may be etiolate even though they would have sufficient nonred light for adequate photosynthesis (Hershy, 2001). Light quality and photoperiod influence the plant hormonal status (Podwyszynska and Gabryszewska, 2003).

Most plant show short growth in white light (daylight fluorescence tubes) which is even more evident in blue and violet light. In red light, plant show a marked stem elongation (Wassink and Stolwijk, 1956). In this experiment, *C. alismatifolia* also showed abnormal elongation under red light. However, the elongation was not significantly different between cool daylight (white light). Similar to tomato, var Vetomold 121, the plants in the yellow and red cabinets also showed conspicuous showed stem elongation which is in contrast to the behavior of plant belonging to the same variety, exposed to white light (Wassink and Stolwijk, 1956).

Although in some plant such as primrose plants, blue light stimulated growth of leaf and elongation of leaf petiole whereas the effect of red light was the opposite (Michalczuk and Goszczynska, 2002).

On the other hand, the pseudo-stem diameter was smaller, the number of plants per clusters and the number of leaves per plant was lower than in natural light treatment but it was not significantly different between red and cool daylight (Table 6.1). Decreasing growth under red light and cool day light was due to lower light intensity (60 µmol $m^{-2}s^{-1}$ PAR) compared with natural light (9,000 µmol $m^{-2}s^{-1}$ PAR). The different light intensity was affective to photosynthesis and metabolism in plant. In moderate light intensity, the plant generally bears longer internodes. However, in some plant such as *Panicum maximum* it was registered that higher light intensity stimulated growth, tillerling and yield (Deinum *et al*., 1996).

C. alismatifolia grown under red light and cool day light condition began to flower early at 4 weeks after planting (4 WAP) (Fig. 6.1A,C). Plants under red light reached dormancy stage at 19 WAP, and earlier than plants grown under cool day light and natural light (23 and 25 WAP, respectively). In potted primrose plants (*Primula acaulis* 'Corona Scarlet') the red light illumination during the entire experiment caused a decrease in the number flower (Michalczuk and Goszczynska,

2002). In addition, flower of *Arachis hypogeal* grown in red and yellow light did not open at 20^{o}C but at 26^{o}C, they were found to open in all spectral regions (Wassink and Stolwijk, 1956). Indicating that the response of plant to spectral quality depend on genetic and other environmental factors.

Under red light treatment, the number of new rhizomes per cluster was 1.00 rhizome and it was not significantly different from those under cool day light (2.00 rhizomes per cluster). Under natural light treatment, the new rhizome had 3.67 rhizomes per cluster (Table 6.2). Lower formation of new rhizomes under red light and cool day light treatments may be due the decrease of photosynthate in plant. The response of red light also depends on seasonal and genotype difference. In rose 'Sabrina', red light promoted root formation during planting in March, May and November, increasing the growth of shoots and roots system quality while in gerbera, it improved rooting only in winter (Podwyszynska and Gabryszewska, 2003).

Photosynthetic rate was measured by measuring the volume of carbondioxide with the LCA-4 instrument at 10.00 am at 5 WAP. The general competence in photosynthetic rate of plants depended on the type of the plant and environment of the plant growth (Jone and Lazenby, 1988). In this experiment, leave areas of plants under natural light conditions was higher than of plants under red light. The leave area was important for photosynthesis. It was found out that the larger leave area gives higher photosynthesis (Nivut, 1992). The leaves area under controlled treatment was larger than under red and cool light treatments (Table 6.1) and brought about by the highest photosynthetic rate as showed in Table 6.3. Not only the leave area, but light quality itself affected the photosynthetic rate. Photosynthesis of plants under blue and red light were more efficient than light in the yellow-green region. However, in *Sinapis alba* found a reduction in photosynthetic efficiency both in blue and green, as compared to red (Wassink and Stolwijk, 1956).

Kim *et al*. (2003) reported that net photosynthetic rate of *in vitro* chrysanthemum was highest under red/blue LEDs followed by florescence and lowest under blue and far-red LEDs and blue light. In *C. alismatifolia,* red and cool day light (fluorescence lamp) reduced the photosynthetic rate compared with those under controlled treatment (Table 6.3, Fig. 6.3).

The chlorophyll fluorescence of *C. alismatifolia* leaves grown under light conditions at 5 WAP shown in Table 6.4. The *Fv/Fm* ratio indicated the effect of outside factors of chlorophyll efficiency and stress of plant (Flagella *et al*., 1994). Generally, the *Fv/Fm* ratio was found in a remarkably narrow range (0.83 ± 0.004) among leaves of many different species, environmental stress the affect PSII efficiency lead to decrease in *Fv/Fm* (Krause, 1991). In this experiment, the *Fv/Fm* of plants grown under red light and cool day light were not decreased compared with those under natural light conditions indicating that plants under these conditions did not stress.

Although, the total chlorophyll, chlorophyll a and chlorophyll b of plants grown under red light were lower than under other treatments (Table 6.4), but high correlation was not obtained between the chlorophyll content and the photosynthesis rate (Marini, 1986). Furthermore, the losses in chlorophyll content are associated with environmental factors (Hendry and Price, 1993). Lower chlorophyll content of plant grown under red light was also found in leaves of tobacco (HongZhi *et al*., 1998).

A decreasing of TNC and starch effect by red light may be involved to enzyme activity for starch degradation. Photoregulated enzyme can be found in starch degradation, pigment synthesis etc. and the effects of light on an enzyme are often described as photomodulatory (Hart, 1988).

Total soluble sugar (TSS) of new rhizome and storage roots under red light was not significantly different from cool day light condition. But, those concentrations under both conditions were lower than under natural light conditions (Table 6.6). This may be due to red light inhibited the translocation of photosynthates out of leaves (Saebo *et al*., 1995), therefore the accumulation of carbohydrates in storage organs were reduced under red light. Total soluble sugar and reducing sugar in plants indicated the mobilization of assimilated to storage organ. It has been considered that the increase of assimilates in the storage tissue might act as this signal for stimulating storage organ development (Thomas and Vince-Prue, 1993). In tobacco, red light increased reducing sugar content in leaves compared with blue light (HongZhi *et al*., 1998). In *C. alismatifolia*, red light decreased reducing sugar in storage organs (both rhizome and storage roots). This means that the translocation of reducing sugar from leaves to storage organ was inhibited.

Ruamrungsri *et al*. (2001) reported th at rhizome of *C. alismatifolia* is the principal organ for N storage and the storage root is the major organ for carbohydrate storage, such as starch and soluble sugar. Increasing of nitrogen compound in storage roots caused by N application, and this compound may play an important role in the storage of N in the storage roots (Ohtake *et al*., 2006). In the present experiment, nitrogen concentration in new rhizome under all treatments was higher than in storage roots, moreover, red and cool day light reduced nitrogen concentration in both organs (Table 6.7). Indicating that red and cool day light affected nitrogen uptake in this plant. In tobacco, red light decreased nitrogen metabolism compared with blue light (HongZhi *et al*., 1998).

The rhizome was composed of high carbohydrate and phenolic compounds. The Phenol/SDS method failed to yield high-quality RNA because it was contaminated with polysaccharides and polyphenol compounds. The same result was obtained by Hosein (2001). After RNA extraction was cleaned up with RNeasy© Kit, it was sufficient for removing the contaminants. The combined protocols gave high yield and quality RNA. Based on the result of the present study, significant alterations of patterns of four polymorphic bands were differentially expressed in the rhizome grown under different light sources.

6.5 Conclusion

C. alismatifolia. grown under red and cool day lights with 60 µmol $m^{-2}s^{-1}$ PAR light intensity caused abnormal elongation of plants compared with control. These conditions accelerated flowering, decreased the photosynthetic rate and chlorophyll contents but increased chlorophyll fluorescence. Illumination of plants during the entire experiment with red or cool day light at 60 µmol $m^{-2}s^{-1}$ PAR decreased yield and quality of rhizomes. Red light reduced the accumulation of TNC, starch and reducing sugar in the rhizome and storage roots. Fructose, glucose and sucrose in new rhizome and storage roots were lower in red light compared with cool daylight and natural light condition. Red light was also inhibited nitrogen accumulation in rhizome and storage roots. Differential gene expression was found by a total of four polymorphic bands when plants were grown under different light spectrum.