Chapter 4

Effect of photoperiod on flowering of *Curcuma alismatifolia* Gagnep.

4.1 Introduction

Environmental conditions and developmental regulation could have pronounced effects in controlling flowering of most plants (Mouradov *et al*., 2002). One of the most important factors controlling flowering time is the duration of the daily light period or photoperiod. Photoperiodic flowering is not only a photoperiodic phenomenon, but also a photomorphogenetic one. While flowering time control is the major effect of photoperiod detection in plants, photoperiod seems to influence some of the later stages of flower development in many plant species. After the induction of floral primordia, the flower meristem is established and produces floral organs. Changes in photoperiod are known to cause detaining of floral primordia, male sterility, floral reversion, and changes in sex expression in a variety of plant species (Thomas and Vince-Prue, 1997). In *Tanacetun cinerariaefolium* L., daylength has a quantitative effect on both flower initiation and development, with both processes are promoted by long-day (Brown, 1992). In *Saccharum spontaneum* L., flowering is controlled by photoperiod. The earliest stage of development, 'induction' and 'initiation of the inflorescence axis primordium' (IAP) are optimally promoted under intermediate days of 12 hrs 30 min, while the subsequent stage 'initiation of inflorescence branch primordia' (IBP) is inhibited by days longer than 13 hrs. The following stage 'initiation of spikelet primordia' (ISP)

shows a quantitatively intermediate response with an optimum photoperiod of 9 hrs to 11 hrs. The elongation of the differentiated inflorescence is found to be slightly sensitive to photoperiods of 13 hrs or longer in one of the clones. Unfavourable photoperiods at stages following induction result in the arrest or delay of inflorescence development, and when these are given during the IAP and IBP stages, reversion to the vegetative condition is commonly occurred (Julien**,** 1973). *Curcuma alismatifolia* flowers in July to August (12-13 hrs of day duration) and goes to dormancy during winter (Nov to Dec) when sunshine duration is approximately 10 hrs. How its flowering behavior is affected by photoperiod remains unknown. Usually, flowering of *Curcuma* begins with floral initiation and then accelerates and sustains rapid elongation of the flower stalk. This experiment aimed to study the effects of photoperiod on growth, flowering and some biochemical accumulation in *C. alismatifolia*. Investigation of the control of floral initiation and development by photoperiod was expected to be useful information for cut flower production, especially for the off-season flowering of this plant.

4.2 Materials and methods

Rhizomes of *C. alismatifolia* Gagnep. of 1.8-2.2 cm in diameter and each with 4 storage roots were planted in 6x8 inch of plastic bags using media containing soil : sand : rice husk ratio 1:1:1 (by volume). After planting, plants were placed in three growth chambers (Growth Chamber, Tissue growth chamber, Contherm Model 620 RHS P6:R with Stabilizer) which set at different photoperiods; i.e. 6, 10, and 14-hrs per day. All the other environmental parameters were kept constant in all treatments, including light intensity was set at 270 µmol photosynthetic photon flux,

70-80% relative humidity and 28 ◦C temperature. The experimental design was a completely randomized design with 10 replications per treatment. The plants were watered as required and nutrients were added to the soil mixture so that each pot contained 150 mg of available N, 150 mg of available P and 150 mg of available K.

Data collection

Plant height, leaf length, leaf width, number of leaves per plant, number of plants per cluster, leaf area, dry weight of leaves, rhizome, storage roots and fibrous roots were determined. Leaf color was measured using chlorophyll meter (Spad-502; Minolta CO.,LTD) at the first floret opening.

Nitrogen and carbohydrate analysis

Leaves, rhizome and storage roots from five plants of each treatment were sampled to determine for the concentrations of soluble, non-soluble nitrogen, total nitrogen (Ohyama *et al.*, 1985; 1986) and total nonstructural carbohydrates content (Smith *et al.*,1964).

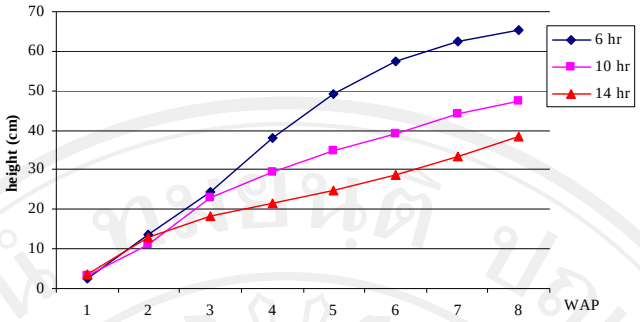
4.3 Results and discussion

Growth of plant

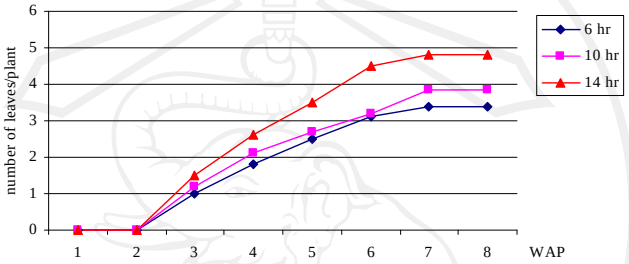
In this experiment, the results showed that plant height and number of leaves per plant grown at the 6 hrs photoperiod were greater than the other treatments (Fig 4.1a, b). However, such plant was taller and slimmer than the other treatments (Fig. 4.2). The plant height at 6 hrs photoperiod tended to increase greater than the other

treatments during 2-8 weeks after planting (WAP) (Fig. 4.2a). The number of leaves per plant at 14 hrs photoperiod also tended to be greater than the other treatments (Fig. 4.1b). During flowering (8 WAP) the plant grown at 14 hrs photoperiod gave the highest number of plant per cluster. Photoperiod did not significantly affect the number of leaves per plant, leaf width or leaf color (Table 4.1). In contrast, Kuehny, *et al*., (2006) reported that *Curcuma alismatifolia* 'Chiang Mai Pink", *C. gracillima* 'Violet' and *C. thorelii* grown in a greenhouse under 16 and 20 hrs photoperiods were taller than those under 8 and 12 hrs. The different result may be caused by the different of light intensity use. Chidburee (2008) reported that under low light intensity at 60 µmol $m^{-2}s^{-2}$, plant height of *C. alismatifolia* at 13 hrs photoperiod was taller than those of 10 and 7 hrs. On the other hand, the height of plant grown under natural condition (high light intensity) at about 13 hrs was shorter than plant grown at 13 hrs under low light intensity.

Figure 4.1 *C. alismatifolia* grown in 6, 10 and 14 hrs photoperiod from left to right.



a)



b)

Figure 4.2 Plant height (a) and number of leaves per plant (b) of *C. alismatifolia* from various treatments.

Table 4.1 Growth of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	Plant height (cm)[1/]	Number of leaves per plant[2/]	Leaf width (cm)[2/]	Leaf length (cm)[1/]	Number of plants per cluster[1/]	Total leaf area[1/]	Leaf color[2/]
6	69.40 a	3.50	5.67	42.00 a	1.30 b	499.60 a	39.74
10	48.20 b	3.40	5.35	31.10 b	1.80 b	307.00 b	38.21
14	39.95 c	4.10	6.18	26.75 c	2.10 a	484.20 a	39.14
$LSD_{.05}$	5.09	ns	ns	3.79	0.45	100.39	ns

[1/] Values within columns followed by different letters were significantly different at $P<0.05$.

[2/] ns: not significantly different

Photoperiod affected dry weight of *C. alismatifolia.* The results showed that growth of plant, in terms of, dry weight was also greatest in new rhizome and storage roots grown at 14 hrs photoperiod. However, the dry weight of old rhizome and storage roots did not affect by photoperiods applied. Dry weight of new rhizome and fibrous roots at 14 hrs were higher than at 6 hrs. In some plants, such as *Lysimochia congestiflora* photoperiod did not significantly affect dry weight (Zhang *et al*., 1995). In *Achillea millefolium* 'Summer Pastels', photoperiod did not significantly affect shoot dry weight (Zhang *et al*., 1996). On the other hand, dry weight of tomato plants increased by 30% when light periods were increased from 12 to 18 hrs. However, extended photoperiod did not increase shoot dry weight of pepper plant (Dorais, *et al.,* 1996). The different responses of dry weight by photoperiod may be due to the the plant grown at 14 hrs photoperiod did not have new storage roots, yet at first inflorescence and the plant grown at 6 and 10 hrs would already have new storage roots with 3.94 and 4.01 g/plant, respectively (Table 4.2). This indicated that the longer photoperiod at 14 hrs delayed storage roots formation of this plant. On the otherhand, the short day at 6 and 10 hrs stimulated early storage roots formation.

Flowering

Changes of day length (photoperiod) functioned as the timer and trigger that activated or stopped physiological processes initiating growth and flowering and activated the process of hardening for resistance to low temperatures in the fall and winter. Vegetative growth was triggered by photoperiod and temperature and reproductive initiation was triggered primarily by photoperiod but could be slightly modified by temperature and precipitation. Cool- and warm-season plants responded

Table 4.2 Dry weight of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	Dry weight (g)						
	Leaves[1/]	Old-Rhizome[2/]	New-Rhizome[1/]	Old-Storage roots [2/]	New-Storage roots	Fibrous root[1/]	Total[1/]
6	3.42 a	0.77	0.58 b	3.18	3.94	0.61 b	18.95 a
10	2.13 b	0.66	0.79 a	2.12	4.01	0.64 b	16.42 a
14	3.95 a	0.59	0.63 ab	2.34	NR[3/]	1.64 a	11.58 b
$LSD._{05}$	0.97	ns	0.17	ns	-	0.36	4.44

[1/]Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

[3/]NR, no new storage roots

to changes in photoperiod differently. Generally, most cool-season plants were long-day plants, and most warm-season plants were short-day plants. Long-day plants reached the flowering stage after exposure to a critical photoperiod and during the period of increasing daylight between the beginning of active growth and mid June, usually flowering before 21 June. Short-day plants were induced into flowering by day length that was shorter than a critical daylength and that occurred during the period of decreasing day length after mid June, usually flowering after 21 June. Short-day plants were technically responding to the increase in the length of the night period rather than to the decrease in the day length (Llewellyn, 2001). A quantitative long day plant, showed a particular day length accelerates but was not essential for flowering (Thomas and Vince-Prue, 1997), which also occurred in *C. alismatifolia.*

Plants grown under 6, 10 and 14 hrs photoperiods produced flower by 20, 30 and 100%, respectively (Table 4.3). Although flower formation could be occurred in all treatments, but only some plants could reach the anthesis stage. Because *C. alismatifolia* was a quantitative long day plant (Hagiladi *et al*., 1997a), therefore the flowering percentage was delayed under short day (6 hrs) conditions. Zhang *et al*., (1995) reported that *Lysimachia congestiflora* Hemsl. was a quantitative long day plant. Plant given a long day photoperiod (16 hrs) flowered at 21 and 34 days earlier than plants given 12 and 8 hrs photoperiods, respectively. Plant under long day produced significantly more flowers than those under 8 and 12 hrs photoperiods and only 1 week of long day was needed for 100% flowering. *Achillea millefolium* 'summer pastels' grown under 16 hrs photoperiod flowered after 27 days, while those under 8 hrs remained vegetative (Zhang *et al*., 1996). Number of pink bracts and spike length of plant grown at 10 and 14 hrs photoperiod were greater than plant grown at 6 hrs. However, it was indicated that short day (6hrs) reduced flower quality, in terms, of number of pink bracts, spike length and spike width. The photoperiod did not significantly affect either number of green bract or spike stalk length. These results suggested that the decrease in flower quality when these plants were grown in winter off-season production, perhaps, mainly due to the shortening of photoperiod.

Nitrogen and total non structural carbohydrates (TNC) contents in plant organs

Leaf

Nitrogen fraction and TNC were analysed at flowering stage. The ethanol-soluble nitrogen fraction was amino acids and peptides, while 'insoluble' fraction was proteins and nucleic acids (Racusen and Foote, 1963). The insoluble nitrogen and

Table 4.3 Flower quality of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photo-period (hrs)	Status of flowering (%)	Number of green bracts[2/]	Number of pink bracts[1/]	Flower stalk length (cm) [2/]	Spike length (cm) [1/]	Spike Width (cm) [1/]
6	Tight flower bud (80) Full bloom (20)	8.33	5.00 b	52.50	10.00 b	3.75 b
10	Tight flower bud (70) Full bloom (30)	8.00	8.33 a	45.00	13.67 a	4.67 ab
14	Full bloom (100)	7.80	7.80 a	45.20	13.20 a	5.80 a
$LSD_{.05}$	-	ns	1.05	ns	1.05	1.45

[1/]Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

total nitrogen in leaf of the plant grown at 6 hrs photoperiod were higher than in plants grown at 10 and 14 hrs (137.80 mg/plant and 140.69 mg/plant, respectively). The photoperiod did not significantly affect soluble nitrogen (2.71 – 3.94 mg/plant), TNC (123.49 - 196.26 mg/plant) or C:N ratio (0.98:1 – 3.51:1) in leaf (Table 4.4). Ruamrungsri *et al*., (2006) revealed that total nitrogen in leaves at flowering stage was derived from original-N (N from mother rhizome) and absorbed-N (N from nutrient solution).

The different of total -N content in this experiment indicated that the short photoperiod at 6 hrs increased absorbed-N in leaves and the photoperiod of 10-14 hrs did not affect nitrogen translocation to leaves. Moreover, the increment of nitrogen in

leaves under 6 hrs was insoluble fraction, it was indicated that most absorbed-N in leaves was assimilated into protein. Similar result was found in poplur (*Populus deltoids*) which short day promoted, wheather directly or indirectly, the accumulation of bark storage proteins by induced rapid changes in gene expression (Coleman *et al*., 1991).

Table 4.4 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in leaves of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [2/]	C:N ratio [1/]
	Insoluble [1/]	Soluble [2/]	Total [1/]		
6	137.80 a	2.89	140.69 a	139.16	0.98:1
10	55.49 b	2.71	58.18 b	196.26	3.51:1
14	69.61 b	3.94	73.56 b	123.49	1.69:1
$LSD_{.05}$	14.12	ns	16.18	ns	ns

[1/]Values within columns followed by different letters are significantly different at $P<0.05$.

[2/]ns: not significantly different

Old rhizome

Ruamrungsri *et al*., (2006) reported that nitrogen content in old rhizome at flowering stage was derived from original-N and absorbed-N. The photoperiod did not significantly affect fraction of insoluble nitrogen (17.02 – 31.92 mg/plant), soluble nitrogen (0.98 – 1.98 mg/plant) or total nitrogen (18.86 – 33.91 mg/plant) in old rhizome (Table 4.5). Different from the influence of photoperiod on the accumulation

of allantoin (soluble-N fraction) in comfrey (*Symphytum officinale* L.), photoperiod promoted an increment in the average content of allantoin in roots. On the other hand, in rhizomes, allantoin accumulation decreased when the photoperiods were increased from 8 to 12 and 16 hrs (Castro *et al*., 2001). The TNC (10.73 – 19.31 mg/plant) and C:N ratio (0.35:1 – 1.12:1) were also not significantly different among treatments (Table 4.5).

Table 4.5 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in old rhizome of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [2/]	C:N ratio [2/]
	Insoluble [2/]	Soluble [2/]	Total [2/]		
6	31.92	1.98	33.91	10.73	0.35:1
10	17.02	1.83	18.86	19.31	1.12:1
14	20.27	0.98	21.26	19.08	0.94:1
$LSD_{.05}$	ns	ns	ns	ns	ns

[2/]ns: not significantly different

Old storage roots

The photoperiod did not significantly affect insoluble nitrogen (70.45 – 117.63 mg/plant), soluble nitrogen (3.94 – 6.15 mg/plant) or total nitrogen (74.97 – 123.79 mg/plant) in storage roots. However, TNC tended to increase when plant was grown under 14 hrs of daylength (34.63 mg/plant) (Table 4.6). In white clover (*Trifolium repens* L.), a shorter photoperiod caused a marked decrease in TNC content

of the leaves (-78%), but only slightly depressed reserve accumulation in stolons and roots (-14%) (Boller and Nösberger, 1983).

Table 4.6 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in old storage roots of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [2/]	C:N ratio [2/]
	Insoluble [2/]	Soluble [2/]	Total [2/]		
6	117.63	6.15	123.79	210.63	2.14:1
10	70.45	4.52	74.97	300.69	4.60:1
14	116.19	3.94	120.14	342.63	2.84:1
$LSD_{.05}$	ns	ns	ns	ns	ns

[2/]ns: not significantly different

New rhizome

Most of nitrogen content in new rhizome and new storage roots derived from N absorbed during flowering to dormancy stage (Ruamrungsri *et al*., 2006). The photoperiod did not significantly alter insoluble nitrogen, soluble nitrogen or total nitrogen in new rhizome of *C. alismatifolia* (Table 4.7). TNC and C:N ratio in the new rhizome of plants grown at 14 hrs photoperiod were predorminantly higher by 38.85 mg/plant and 2.18:1, respectively than those in plant grown at 6 and 10 hrs. Indicating that carbon from photosynthesis was predorminantly used for starch synthesis in new rhizome but not used for protein synthesis. Therefore, it brought about by the significantly increase of TNC, however, it was not significantly different

in insoluble-N fraction in new rhizome of plant under 14 hrs of photoperiod. On the contrary, short photoperiod (6 hrs) tended to increase insoluble-N and total-N fractions. The similar result was found in *Medicago saliva* in which short day treatment resulted in preferential partitioning of N to taproots (Noquet *et al*., 2003).

Table 4.7 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in new rhizome of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [1/]	C:N ratio [1/]
	Insoluble [2/]	Soluble [2/]	Total [2/]		
6	20.99	0.35	21.35	2.98 b	0.14:1 b
10	15.44	0.05	15.49	8.26 b	0.50:1 b
14	18.53	0.06	18.60	38.85 a	2.18:1 a
$LSD_{.05}$	ns	ns	ns	4.68	0.26

[1/]Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

Spike

The photoperiod did not significantly affect C:N ratio (1.51:1 – 2.17:1) in spike. Insoluble, total nitrogen and TNC in spike of plant grown in 10 hrs and 14 hrs were the highest when compared with the other treatment. Soluble nitrogen of plant grown under 14 hrs of daylength was the highest (1.37 mg/plant) when compared with the other treatments (Table 4.8).

Table 4.8 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in spike of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [1/]	C:N ratio [1/]
	Insoluble [2/]	Soluble [1/]	Total [2/]		
6	1.38 b	0.06 b	1.45 b	2.13 b	1.51:1
10	6.54 a	0.70 ab	7.24 a	16.34 a	2.32:1
14	6.22 a	1.37 a	7.59 a	15.81 a	2.17:1
$LSD._{05}$	1.87	0.88	1.98	3.67	ns

[1/]Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

Whole plant

Insoluble nitrogen and total nitrogen concentrations of the whole plants that grown at 6 hrs photoperiod were 309.74 and 321.20 mg/plant, respectively; and were significantly higher than those in plant grown at 10 and 14 hrs. On the other hand, TNC content and C:N ratio of the whole plants which grown at 14 hrs photoperiod tended to increase when compared with those in 6 hrs treatment (Table 4.9).

Carbon metabolism is inextricably linked to nitrogen metabolism and any effect of change in carbon abundance would have impacts on nitrogen metabolism and *vice versa*. Utilization of sucrose in growing sinks depends on the simultaneous provision of amino acids. The amino acids synthesis also requires ATP, reductants and carbon skeletons. Where they are provided by photosynthesis, glycolysis and respiration (Paul and Foyer, 2001). The increase in flowering percentage, number of

pink bracts, spike length and spike width by long photoperiod (14 hrs) in this experiment might be related to the increase of TNC in plant (539.86 mg/plant) (Table 4.9). Chidburee (2008) reported that the photosynthetic rate of *C. alismatifolia* grown under 13 hrs was significantly higher than those grown under 7 and 10 hrs during 3-4 WAP. Moreover, total chlorophyll content, chlorophyll a and chlorophyll b of plant grown under 13 hrs at 5 WAP were also higher than those in plants grown under 7 hrs. Therefore, it can be concluded that the increase in photosynthetic rate under long daylength was probably brought about by the increase in photosynthates (TNC) which plants used for promoting flowering.

Table 4.9 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC and C:N ratio in whole plant of *C. alismatifolia* under different growing photoperiods at 12 WAP.

Photoperiod (hrs)	nitrogen (mg/plant)			TNC (mg/plant) [2/]	C:N ratio [2/]
	Insoluble [1/]	Soluble [2/]	Total [1/]		
6	309.74 a	11.45	321.20 a	363.64	1.14:1
10	164.93 c	9.83	174.77 c	540.87	3.03:1
14	230.84 b	10.31	241.16 b	539.86	2.23:1
$LSD_{.05}$	43.18	ns	46.04	ns	ns

[1/]Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

4.4 Conclusion

Short day treatments increased plant height and leaf length, but decreased the number of plants/cluster. Long day treatments increased dry weight accumulation in actively growing plant parts; i.e. new rhizome, and fibrous roots; and leading to promote quality of spike and C:N ratio of plant. Plants could flower under short day condition (6 and 10 hrs) but the development of flower was delayed when compared with long day condition (14 hrs). Short photoperiod (6 hrs) increased the insoluble-N fraction but decreased TNC in plant. Long photoperiod (10 and 14 hrs) increased the insoluble-N fraction, soluble-N fraction, Total-N and TNC in spike.