Chapter 5

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

5.1 Introduction

Flowering process is controlled by environmental conditions and developmental regulation, where temperature is the major factor that affects flowering of flower bulbs (Mouradov *et al*., 2002., De Hertogh and Le Nard, 1993). In many bulbs, the process of organogenesis inside the bulb during the rest period, growth, and flowering is temperature dependent. Not only flowering, temperature affects the rates of all plant processes which most physiological processes go on normally in plant range from approximately 0°C to 40 °C. Very high and very low temperatures can cause injury effect to plant (Kamenetsky *et al*., 2000, Went, 1953).

The effects of temperature on the individual life and growth processes must be known in order to understand the effect of temperature on plants growth and development. The growth of excised plant parts has been studied at different temperatures. The optimal growth temperatures for tomato roots was found to be 30 °C; for cotton roots was 25 °C; for corn and sunflower roots was 20 °C and for pea roots was 10°C. For callus tissue, these values were 24 °C to 28 °C for sunflower, and 32 °C for tobacco (Went, 1953).

The ecological and biological factors which may influence flowering of caraway were investigated. Temperature and the length of the induction played a significant role in flower initiation. Among the examined variations, seven weeks of

cold temperatures (8 °C day and 5 °C night) proved to be optimal for flowering (100%). Shorter periods of cold temperatures (1 -2 weeks) or higher temperature regimes (15 °C day and 10 °C night) induced only partial flowering (18-80%) (Németh *et al*., 1997).

In apple, nitrogen (N) uptake by apple roots began about 3 weeks after bud break. Rate of uptake of ^{15}N increased with increasing soil temperature and changed with plant growth stage. Before bud break, ^{15}N was not detected in trees growing in the 8 °C soil treatment, whereas ^{15}N uptake increased with increasing soil temperatures between 12 and 20 °C. Ten days after bud break, ^{15}N was still not detected in trees growing in the 8 °C soil treatment, although total ^{15}N uptake and uptake rate continued to increase with increasing soil temperatures between 12 and 20 °C. Twenty-one days after bud break, trees in all temperature treatments were able to acquire ^{15}N from the soil, although the amount of uptake increased with increasing soil temperature. Distribution of ^{15}N in trees changed as plants grew. Most of the ^{15}N absorbed by trees before bud break (~5% of ^{15}N supplied per tree) remained in the roots. Forty-six days after bud break, approximately one-third of the ^{15}N absorbed by the trees in the 12–20 °C soil temperature treatments remained in the roots, whereas the shank, stem and new growth contained about two-thirds of the ^{15}N taken up by the roots. Total amino acids concentration and distribution of amino acids in trees changed with plant growth stage, but only the amino acids concentration in new growth and roots was affected by soil temperature. It was concluded that a combination of low soil temperature and plant developmental stage influenced the ability of apple trees to take up and use N from the soil in the spring (Dong *et al*., 2001).

Went (1953) reported that in many cases, plants grown at a low temperature had higher sugar content indicated that translocation decreased linearly with increase in temperature from 2 °C upward.

C. alismatifolia called “Chaing Mai Pink”, which has a lilac-purple lotus-like flower, terminal spike is very prominently high above the leaves, making it very suitable for cut flowers. The colored bracts are arranged resemble to a lotus flower, in the way that curcuma lovers in the Netherlands view it as a tulip (Vichailak, 2006).

Cut flowers and rhizomes of *C. alismatifolia* are exported to many countries such as Japan, U.S.A, the Netherland and the New Zealand (Pubuwpern, 1992). In 2004, Thailand exported 1,233,581 rhizomes to the USA., Australia, Ecuador, Israel, Italy, Japan, Korea, Nepal, the Netherlands, Pakistan and Singapore (Department of Agricultural Extension, 2005). After arrival, rhizomes are planted in temperature-controlled greenhouse and sold as both cut flowers and flowering potted plants (Vichailak, 2006). Because it is tropical plant, therefore, growth and development of this plant in winter is reported and plant is gone to rest. The experiment aimed to study the effect of constant temperature and day/night temperatures on growth, flowering and food reserve in *C. alismatifolia*.

5.2 Materials and methods

Rhizomes of *C. alismatifolia* Gagnep. each with four storage roots were planted in 6x8 inch plastic bags using media containing soil : sand : rice husk at a ratio of 1:1:1 (by volume). After planting, plants were placed in a growth chamber (Conther phytotron climate simulator) at 28 °C until shoots emerged (about 4 weeks after planting: WAP).

Then plants were transferred into growth chambers (Fig. 5.1) set at constant temperatures of 18, 20, 22, 24, 26 and 28 °C. All the other environmental conditions were kept constants in all treatments, including light intensity was set at 270 µmol photosynthetic photon flux, relative humidity at 70-80% and photoperiod of 12-hrs. The experimental design was a completely randomized design with 10 replications per treatment.

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 measured. Leaf color was measured using chlorophyll meter (Spad-502; Minolta CO.,LTD) at 7 weeks after planting (WAP).

Nitrogen and carbohydrate analysis

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

Figure 5.1 Plants grown in growth chamber under different constant temperatures.

5.3 Results and discussion

Plant growth

The height of plants grown in constant temperature at 28 °C for 7 WAP tended to be taller than the other treatments (Fig. 5.2). Similar result was found in poinsettia cv. Lilo and Starlight which was given a 2-hour temperature drop (from 19 to 13 °C) or temperature increase (from 19 to 25 °C) from the start of short day-treatment until flowering. A temperature drop during the last 2 hours of the night or the first 2 hours of the day reduced plant height, plant diameter and the length of leaf and bract petioles, but had only slight influence on bract size and flower development (Moe *et al*., 1992). In *Cosmos atrosanguineus*, plant height was doubled and flower area was increased as temperature rised from 13 °C to 26 °C (Kanellos and Pearson, 2000).

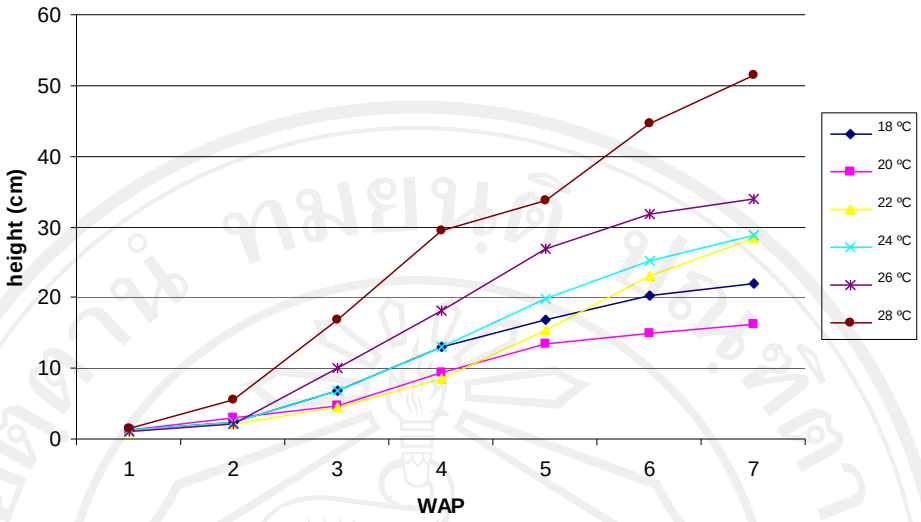

Figure 5.2 Height of *C. alismatifolia* under different growing temperatures during 1-7 WAP.

Plant height at 8 WAP was the shortest at 20 °C treatment. Increasing temperatures could promote plant height from 16.20 cm at 20 °C to 51.40 cm at 28 °C (Table 5.3). Number of leaves per plant at 28 °C was averaged at 4.0 leaves per plant and it was significantly higher than the other treatments. Leaves of plant grown at 18 °C could not fully expand (Table 5.1). Temperature also affected leaves size (width and length). Higher temperatures significantly increased leaves size of *C. alismatifolia*. It was probably dued to low temperature limited some biological reactions and the availability of energy was inadequate (Barbour *et al*., 1987). Different plant species have different optimum temperature ranges. Cool-season plants, which are C3 photosynthetic pathway plants, have an optimum temperature range from 10 to 25 °C. Warm-season plants, which are C4 photosynthetic pathway plants, have an optimum temperature range from 30 to 40 °C (Coyne *et al*., 1995). It is indicated that temperature involves the assimilation of photosynthates and thus

affects growth of plant. Ferraris (2005) reported that during the juvenile phase of *Pennisetum purpureum* Schum, the higher temperature increased the leaf appearance rate, tillering rate and main stem elongation. Harbaugh (1995) reported that 4 cultivars of *Eustoma* seedling were larger and had more leaves when grown at 28 °C than at 12 °C which were similar to those of *C. alismatifolia*. Leaf expansion rate was greatly influenced by temperature where leaf emergence rate and the temperature optimum for leaf expansion were differed for different crops (Jonathan *et al*., 2006). Meriam *et al*., (2006) found that leaf unfolding rate of *Hibiscus rosa-sinensis* 'Brilliant Red' and 'Pink Versicolor' grown at 5 and 11 °C developed chilling injury. At 5 °C, plants did not survive and at 11 °C, plants grew very slowly. The two cultivars unfolded leaves at similar rates over the 11-35 °C temperature range. In this experiment, high temperature also increased leaf color of *C. alismatifolia* (Fig. 5.3, Table 5.1). The data of chlorophyll meter (SPAD unit) of plants grown at 28 °C was significantly higher than the other treatments (Table 5.1). Leaf chlorophyll content was reduced in plant grown with a negative DIF (Berghage *et al*., 1990). Total leaf chlorophyll increased as DIF increased in *Fuchsia* (*Fuchsia* x *hybrida* Hort. Ex Vilm.) and *Dendranthema* (Erwin and Heins, 1995).

Leaf dry-weight in Table 5.2 was also the greatest in plants growing at 28 °C and the least in those plants growing at 18 and 20 °C. Plants grown at 22-28 °C had the highest dry weight of storage roots, on the other hand, plants grown at 18-20 °C gave the least dry weight. The fibrous roots were also the greatest in plants growing at 24-28 °C, followed in order by the 20 and 22 °C. Similar to avocado cv. Fuerte and cv. Hass, in which high temperatures produced maximum dry matter in the leaves, while low temperatures produced it in the roots (Lahav and Trochoulias, 2006).

Table 5.1 Growth of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature (°C)	Plant height (cm)[1/]	Number of leaves per plant[1/]	Leaf width (cm) [1/]	Leaves length (cm) [1/]	Leaf color (SPAD UNIT) [1/]
18	22.03 c	FL[2/]	FL[2/]	FL[2/]	FL[2/]
20	16.20 d	1.4 d	3.04 d	11.25 d	FL[2/]
22	28.45 b	1.5 d	6.50 b	21.27 c	19.58 c
24	28.81 b	2.5 c	4.58 c	21.85 c	24.88 c
26	34.01 b	3.4 b	5.13 c	24.89 b	35.32 b
28	51.40 a	4.0 a	7.47 a	30.90 a	50.10 a
$LSD_{.05}$	5.76	0.53	0.83	2.67	7.11

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

[2/]FL, folded leaf.

However, temperature did not significantly affect rhizome dry weight (Table 5.2). The accumulation of dry matter in different plant parts was related to the partitioning of photosynthates because photosynthetic activity per unit leaf dry matter increased with increasing temperature (Acock *et al*., 1979). Therefore, temperature influenced plant development, it also influenced partitioning of assimilates to different plant organs. This should be reflected in the effect that temperature has on the mass of individual plant organs (Myster and Moe, 1995).

Table 5.2 Dry weight of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing Temperature (°C)	Dry-weight (g)				
	Leaves[1/]	Rhizome[2/]	Storage roots [1/]	Fibrous root[1/]	Total[1/]
18	0.40 c	1.50	1.32 b	NR	2.53 b
20	0.25 c	1.30	2.07 b	0.04 c	3.66 b
22	1.42 b	1.11	6.53 a	0.41 b	9.46 a
24	1.38 b	1.50	5.35 a	0.77 a	9.00 a
26	1.60 b	1.18	7.96 a	0.90 a	11.65 a
28	2.92 a	1.18	5.60 a	0.71 a	10.41 a
$LSD._{05}$	0.64	ns	2.80	0.22	3.09

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

[2/]ns: not significantly different

Flowering

Temperature influences most plant processes, including photosynthesis, transpiration, respiration, germination and flowering. As temperatures increase (up to a point), photosynthesis, transpiration and respiration also increase. *C. alismatifolia* grown at 28 °C had the percentage of flowering approximately 80%, in contrast, the plants grown at 18-20 °C could not flower (Table 5.3). Plants were stunt and leaves were yellowish. Although plants under 22 °C could produce a young flower bud but it could not develop until anthesis. In flowering process, the leaves must be competent to produce any stimulus that is required by the apex and then the shoot apex must also

be competent to respond and must then become progressively determined as the successive floral organs are formed (Lyndon, 1990). Under 18-20 °C growth of leaves were retard therefore the required stimulus could not be produced and thus flowering of *C. alismatifolia* could not succeed. Depending on the situation and specific plant, the effect of temperature can either speed up or slow down the transition from vegetative to reproductive (flowering) (http://extension.oregonstate.ded/mg/botany/light.htm., 2006). In this experiment, temperature at 24-26 °C delayed flowering of *C. alismatifolia* since at that time the flowering percentage was lower than those plants at 28 °C. It should be noted that this experiment was carried out under constant temperature. Characteristic of growth and development of this plant under the day/night temperature conditions should be further studied.

Table 5.3 Number of plants per cluster, percentage of flowering of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature (°C)	Number of plants per cluster[1/]	Percentage of flowering
18	1.00	NF
20	1.10	NF
22	1.20	NF
24	1.20	50.00
26	1.00	60.00
28	1.00	80.00
$LSD._{05}$	ns	-

[1/]ns: not significantly different

[2/]NF: not initiate flower

a) at 18 °C (left) vs 28 °C (right)

b) at 20 °C (left) vs 28 °C (right)

c) at 22 °C (left) vs 28 °C (right)

d) at 24 °C (left) vs 28 °C (right)

e) at 26 °C (left) vs 28 °C (right)

Figure 5.3 Growth of *C. alismatifolia* under different growing temperatures at 8 WAP.

Nitrogen and total nonstructural carbohydrates (TNC) in leaves

Nitrogen promotes the plant growth by influencing the phytohormonal status of plant and by being an elemental constituent of essential molecules, such as protein and nucleic acids. It is involved in the most important physiological processes for crop production (Mengel, 1992). Marschner (1995) has suggested that the non-protein N (soluble fraction nitrogen), such as amine acids and amides, acted as intermediates between the assimilation of inorganic N and the synthesis or degradation of the high-molecular-weight compounds. There was a strong correlation between the rate of photosynthesis and leaf nitrogen concentration because of the high N investment in the photosynthetic apparatus (Lindsey *et al*., 2007). In this study, insoluble nitrogen and total non structural carbohydrates (TNC) concentration were analysed in leaf. The results showed that insoluble (mainly storage protein-N) and total nitrogen concentrations in leaves of plants grown at 28 °C (approximately 56.90 and 66.40 mg/ plant, respectively) were higher than those in the other treatments. The soluble nitrogen of leaves in plants grown at 24 °C and 28 °C were also higher than the other treatments.

Total non structural carbohydrates (TNC) of the plant are reserves available for growth and respiration. The amount of total nonstructural carbohydrates in a plant is influenced by environment, taxonomy, anatomy, stress, phenology, and management. Diurnal fluctuations in TNC have been documented to occur. An increase in total sugars and starch occurred in a witchgrass (*Panicum virgatum* L.) between 6 am and 6 pm, followed by a drop until midnight. From 6 am to midnight, basal sheaths and internodes (i.e. storage parts) accumulated starch. Nonstructural carbohydrates content of perennial plant parts may be used to determine translocation

of photosynthate (Bewick *et al.*, 1997). In present result, the TNC concentration of leaves were also the greatest in plants grown at 26 °C and 28 °C (i.e. 59.17 and 56.81 mg/plant, respectively). TNC and N in leaves could serve as alternative source of assimilates. The C:N ratio of plant grown at 18 °C were higher than those in the other treatments (Table 5.4). This was indicating that the majority of assimilates in leaf would be TNC rather than nitrogenous compounds.

Table 5.4 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC concentrations and C:N ratio in leaves of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature (°C)	nitrogen (mg/plant)			TNC (mg/ plant) [1/]	C:N ratio [1/]
	Insoluble [1/]	Soluble [1/]	Total [1/]		
18	7.87 cd	0.61 c	8.48 c	25.01 bc	2.73:1 a
20	5.82 d	1.03 c	6.85 c	9.85 c	1.41:1 cd
22	20.36 bc	3.02 bc	23.38 b	47.44 ab	2.10:1 ab
24	18.11 b	9.10 a	27.22 b	43.17 ab	1.64:1 bc
26	25.57 b	7.31 ab	32.89 b	57.17 a	1.73:1 bc
28	56.90 a	9.50 a	66.40 a	56.81 a	0.89:1 c
$LSD._{05}$	12.80	4.70	12.52	23.45	0.86

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

Nitrogen and total nonstructural carbohydrates in rhizome

The plant grown at 24 °C had the highest soluble nitrogen (9.49 mg/plant) while, the plant grown at 18 and 22 °C contained lower than those in the other treatments. The plant grown at 18 °C gave the highest insoluble nitrogen and TNC concentration, i.e. 49.49 and 99.59 mg/plant, respectively. The C:N ratio of plant grown at 18 °C and 20 °C were higher than those in the other treatments (Table 5.5). The constant temperature did not significantly affect the concentration of total nitrogen in rhizome of plant.

Table 5.5 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC concentrations and C:N ratio in mother rhizome of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature(°C)	nitrogen (mg/plant)			TNC (mg/plant) [1/]	C:N ratio[1/]
	Insoluble[2/]	Soluble[1/]	Total[2/]		
18	49.49 a	3.52 c	53.01	99.59 a	1.76:1 a
20	49.43 a	4.25 bc	53.69	65.54 ab	1.31:1 a
22	26.95 ab	1.65 c	28.61	18.03 c	0.66:1 b
24	38.34 ab	9.49 ab	47.83	26.92 bc	0.74:1 b
26	20.22 b	5.39 abc	25.61	14.96 c	0.59:1 b
28	32.19 ab	8.52 a	40.71	28.06 bc	0.63:1 b
$LSD_{.05}$	28.55	4.74	ns	46.47	0.47

[1/]Values within columns followed by different letters were significantly different at $P<0.05$. [2/]ns: not significantly different

Nitrogen and total nonstructural carbohydrates in storage roots

The concentrations of insoluble nitrogen and total nitrogen in storage roots of plant grown at 28 °C were the highest (78.31 and 89.66 mg/plant, respectively). The plants grown at 26 °C gave the highest TNC concentration (184.38 mg/plant). The plants grown at 22 °C had the highest C:N ratio (i.e. 4.93:1) (Table 5.6). The plants grown at 24 °C gave the highest concentration of soluble nitrogen in storage roots. Total nonstructural carbohydrates in roots of Alfalfa (*Medicago sativa* L.) was the highest for the plants grown at 21/8 °C followed in descending order by that of plants grown at 12/2 and 34/25 °C. (Al-Hamdani and Todd, 1990).

Table 5.6 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC concentrations and C:N ratio in storage roots of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature (°C)	nitrogen (g/plant)			TNC (mg/plant) [1/]	C:N ratio[1/]
	Insoluble[1/]	Soluble[2/]	Total[1/]		
18	21.12 cd	2.53 b	23.65 c	83.67 c	3.68:1 ab
20	32.03 bc	8.78 b	40.82 b	107.49 bc	2.79:1 b
22	8.83 d	13.34 ab	22.17 c	103.41 bc	4.93:1 a
24	13.98 cd	23.88 a	37.86 bc	68.67 c	1.89:1 b
26	48.52 b	11.22 ab	59.74 b	184.38 a	3.13:1 ab
28	78.31 a	11.34 ab	89.66 a	157.68 ab	1.83:1 b
$LSD_{.05}$	20.12	13.07	25.66	65.05	1.84

Values within columns followed by different letters were significantly different at $P<0.05$.

[2/]ns: not significantly different

Nitrogen and total nonstructural carbohydrates in whole plant

The concentrations of insoluble nitrogen and total nitrogen in whole plant which grown at 28 °C were the highest (169.41 and 196.98 mg/plant, respectively). The plants grown at 24 °C gave the highest insoluble nitrogen concentration (42.48 mg/plant). The plants grown at 18-22 °C gave the highest C:N ratios (i.e. 2.46:1, 1.89:1 and 2.21:1, respectively) (Table 5.6). The constant temperatures did not significantly affect the concentration of TNC in whole plant. The source-sink relationship is crucial in plant production. The growth rate of sink tissues and organs is limited by photosynthate supplies from source leaves (source limitation). Carbon metabolism and nitrogen metabolism are the two basic metabolism processes in plant development. They directly affect the formation and transformation of the photosynthetic product, mineral nutrition absorption, and protein synthesis. The ratio of carbon and nitrogen affect source-to-sink ratio and crop yield (Hong-Biao *et al*., 2008).

Table 5.7 Insoluble nitrogen, soluble nitrogen, total nitrogen, TNC concentrations and C:N ratio in whole plant of *C. alismatifolia* under different growing temperatures at 8 WAP.

Growing temperature (°C)	nitrogen (g/plant)			TNC (mg/plant)	C:N ratio
	Insoluble	Soluble	Total		
18	78.49 bc	6.66 d	85.15 bc	208.28	2.46:1 a
20	87.29 bc	14.07 cd	101.37 bc	182.89	1.87:1 a
22	56.15 c	18.02 bcd	74.17 c	168.88	2.21:1 a
24	70.43 bc	42.48 a	112.92 bc	138.92	1.31:1 c
26	94.32 b	23.93 bc	118.26 b	256.51	2.15:1 ab
28	167.41 a	29.37 ab	196.98 a	242.56	1.23:1 c
$LSD_{.05}$	36.70	14.54	40.77	ns	0.52

Values within columns followed by different letters were significantly different at $P<0.05$.

WAP, weeks after planting.

5.1 Conclusion

Temperature affected growth of *C. alismatifolia* during vegetative to reproductive (flowering) stage. Temperatures lower than 26 °C reduced plant height, number of leaves per plant, leaf size, leaf color and dry weights. It leaded to the retard of flowering, especially at 18-22 °C. Temperatures in the growing environment of *C. alismatifolia* at 24- 26 °C delayed flowering. High temperatures increased nitrogen fraction and total nitrogen, while decreased C:N ratio in plant. In mother rhizome, high temperature decreased insoluble-N fraction, TNC, and C:N ratio.