

## Chapter 2

### Review of Literature

#### 2.1 Botanical characteristics

*Curcuma alismatifolia* Gagnep. is in the Tribe Zingiberaceae, a member in Zingiberaceae family. This species grows well in the open areas. The nature of this genus is a rhizomatous herbaceous plant, comprising underground parts, leafy shoot leaf blades (Sirirungsa *et al.*, 2007). It has a pseudostem which causes by folded clasping leaf sheaths; has 4-5 lateral buds, simple leaf with lanceolate or ovate shape, green leaf sheath, red base, smooth glabrous leaf and probably its midrib can be red. It has two types of roots: the roots emerging at the very first growth, called fibrous roots, and becomes contractile roots for a period of the development of the plant, as well as, absorbing foods. The contractile roots will be swollen, shaping knob, to store nutrient if dormancy period is coming, then they are called storage roots (Ruamrungsri *et al.*, 2005). An inflorescence grows at an apex of pseudostem in a compact spike; it consists of bract that has two types of bract base - first, pink coma bract, which is longer than lower bract; and second, glossy thick green lower bract. True flower has no pedicel (Figure 2.1). It is composed of three calyx tube sepals and three petals. The flower blooms at the base through the apex (Lekawatana and Pituck, 1998).

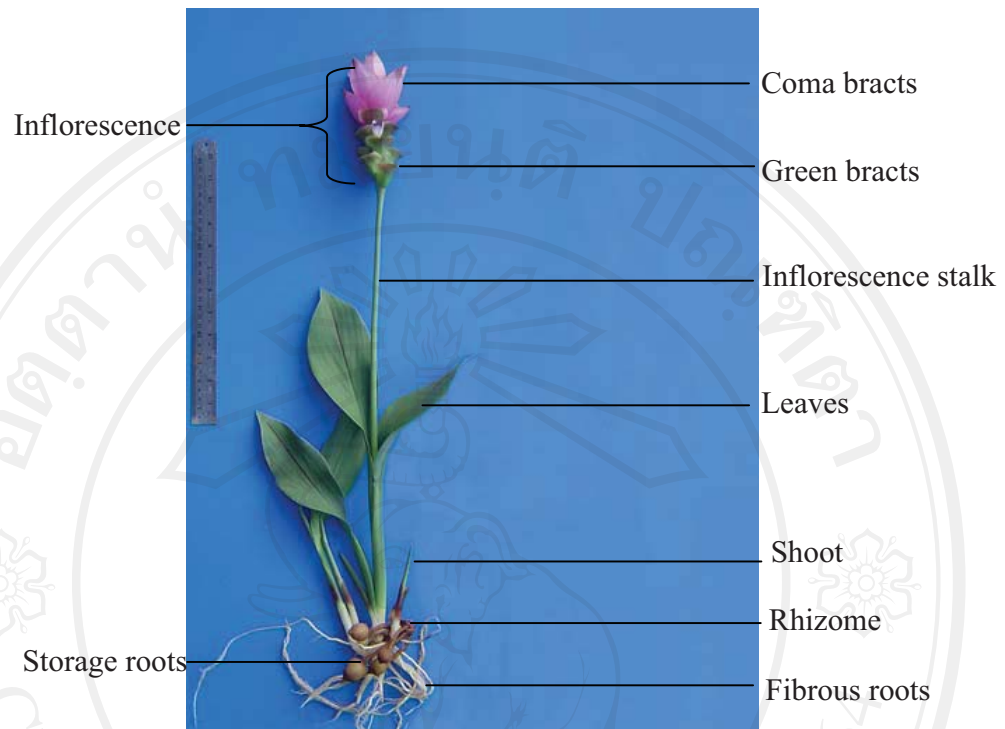


Figure 2.1 Morphology of *Curcuma alismatifolia* Gagnep.

It is a perfect flower that grows around the corner of the bract. In each bract, there are approximately 4 - 6 true flowers. One floret in the same inflorescence blooms in 4 days after another (Pubuopiend, 1993).

## 2.2 Growth of *Curcuma alismatifolia* Gagnep.

*C. alismatifolia* Gagnep. is the perennial ornamental bulb that begins to be grown annually from March to September. *C. alismatifolia* Gagnep. yields flowers during rainy season with 2-3 months of blooming period. It produces the inflorescence in 70 days after growing; thrusts the apex of floret in 90 days after planting; and at the first floret blooms, 105 days after growing, concomitantly the base of stalk begins to swell, and

contractile roots gradually stores water and foods, develops into a knob at the end of the roots. Meanwhile, *C. alismatifolia* Gagnep. continually grows by producing averagely 2 – 5 rhizomes. However, during winter the plant is in its dormancy; the above-ground part of the plant is dried-up, so it's time for rhizome's harvest (Ruamrungsri *et al.*, 2005).

### **2.3 Seasonal production of *Curcuma alismatifolia* Gagnep.**

The species in genus *Curcuma* can be grown in diverse tropical conditions, from sea level to a height of 1500 m on the hilly slopes, at the temperature range of 20 - 30 °C, 12-13 hours of sunshine duration and 80 % RH. A rainfall of 150 mm or more is essential for optimum growth and development. Soil requirements are loose, friable loamy or alluvial suitable for irrigation that should have efficient drainage capacity for growing. There are two types of planting dates; regular season planting (rainy season) and off-season planting (winter season) (Department of Agricultural Extension, 2005).

#### **2.3.1 Regular season planting dates**

Regular season planting can be divided into 3 periods, i.e., (1) Early-season planting (February to March), the rhizome must be soaked in water to stimulate shoot emergence (pre-germinated) before planting. It will flower in May and rhizomes will be sold in December. (2) Mid-season planting (April or May), flowering occurs about 2 -3 months after planting depending on rainfall (July or August) and rhizomes are harvested and sold in December and January. (3) Late-season planting (June to July), rhizome must be carefully kept to avoid drying out before planting, Flowers are produced in September to October. The harvested rhizome has short swollen root, which is the most suitable for export (Wichailak, 2006; Department of Agricultural Extension, 2005).

### 2.3.2 Off-season planting dates

Naturally, when short day condition begins in September and the changes of environmental factors, such as photoperiod and temperature, become unfavorable for *Curcuma*. All of the above ground parts wilt and die down, and rhizomes enter dormancy period until next rainy season. The delayed planting in October for off-season production often decreases plant height, number of shoots per plant, rhizome, flowering percentages and flower quality of this plant (Ruamrungsri *et al.*, 2007). Thus, artificial light for the night break technique has been used to improve the quality of flowers and rhizome for off-season production of *Curcuma*.

*C. alismatifolia* Gagnep. which grown under long day condition using continuous light for 2 hours (supplemental lighting from 08:00 – 10:00 pm) gave the best result, in terms of number of shoots per cluster, number of flowers per plant, number of new rhizomes, size and weight of new rhizomes (Ruamrungsri *et al.*, 2007; Payakaihapon and Ruamrungsri, 2006). Some reports revealed that supplemental lighting increased photosynthetic rate, and maintained a higher photosynthetic rates during the day than the control plant in tomato, lettuce and garland chrysanthemum (Erhioui *et al.*, 2002; Fukuda *et al.*, 2000). Plant under supplemental lighting had increased photosynthesis and starch content in leaf (Gosselin *et al.*, 1996) and the increase of leaf area and dry weight reflected by the increase of photosynthetic area (Cockshull, 1966).

The advantages of the off-season production are to: (1) avoid overage of the regular season cut flower or rhizome production, to avoid danger of epidemics, and to increase income of growers, (2) satisfy customers at the time of their needs; and

(3) guarantee employment throughout the year.

## **2.4 Agricultural practices**

### **2.4.1 Preparation and propagation of *C. alismatifolia* Gagnep.**

#### **2.4.1.1 Rhizome for planting preparation**

The first consideration for planting preparation is to select only the disease-free rhizome, unaffected with nematodes and that comes from disease-free planting fields. The equal size of rhizome should be selected for planting in the field. Generally, the rhizomes are graded according to size, as follows: large, with diameter larger than 1.5 cm; medium, with a diameter of 1.0 - 1.5 cm and small, with a diameter less than 1.0 cm. The selected rhizomes are placed in germination boxes with planting materials, such as sand, rice husk, charcoal and coconut dust. The growing conditions should be maintained with the partial sunlight and 70% relative humidity to stimulate sprouting (Department of Agriculture, 2006 and Wichailak, 2006).

#### **2.4.1.2 Planting area and soil preparation**

The land to be planted with curcuma should not be contaminated with rhizome rot or wilt disease and must be free of nematodes. On land, where plants that are known as to host diseases affected on curcuma should have never been planted for at least two years. Land, on which curcuma has never been planted previously should be selected. If land has been planted with curcuma, it should be rotated with other crops for at least three years before returning to curcuma again. The soil should be sandy loam, with medium fertility, well-drained and having soil pH of 6.5-7.0. Avoid planting in the

alkaline soil, by adding sulfur, as curcuma will be stunted and leaves become yellow with pale flower which is caused by the macro nutrient deficiency (Department of Agriculture, 2006; Wichailak, 2006).

There are two ways of planting curcuma. (1) Field planting: plows the land once and leaves it to be exposed to the sunlight for 20-30 days. As a preventive measure against rhizome rot disease, the urea mixed with lime at the ratio of 1:10 is applied to the soil at the rate 62,500 rhizome per hectare before harrowing. The land is bedded, covered with plastic sheet and left for 15 days. The beds are sub-divided into small plots of about 400 m<sup>2</sup> and water drainage channels are provided. Within the sub-division, the beds are raised the level by 20-30 cm high and 1.0-1.2 m wide, provided a 0.5 m wide path for walking space and a 1 m wide between the sub divisions. The soil should not be plowed too deeply as the storage roots will be grown down too deep, rhizome would get damaged when harvest and become undesirable for the market. (2) Plastic bag planting: a potting material mixture (sand : rice husk or coconut coir dust : rice husk charcoal at a ratio of 1:1:1) is prepared and filled into a black plastic bag sized 15 x 30 cm, bags are then placed on a sheet of clear plastic on a raised planting platform of 20 cm high (Department of Agriculture, 2006; Wichailak, 2006).

#### **2.4.1.3 Planting preparation**

In the field planting method, planting space is depended on the sizes of the rhizome, i.e. large (30x30 cm or 62,500 rhizome per hectare), medium (25x25 cm or 93,750 rhizome per hectare) and small (20x20 cm or 125,000 rhizome per hectare). Approximately 15 g of 15-15-15 or 16-16-16 fertilizer is placed in the bottom of the

planting pit before planting, then one rhizome is placed at 7-10 cm deep; after which it is covered with soil and topped with straw or plastic sheet to preserve moisture. For plastic bag planting: planting the sprouted rhizome close to the soil surface with upright shoot, this will result in inducing flowers about two weeks earlier than usual. After that, covers it with soil thinly in order to protect the shoot from burning (Department of Agriculture, 2006; Wichailak, 2006).

#### **2.4.2 Water and fertilizer application**

The water should be clean with pH in the range of 5.5-6.5, without contamination from any organic matters or toxic inorganic matters.

Field planting: after the first pair of opened leaves, a high nitrogen fertilizer should be applied around the plant, such as 21-7-14, 15-0-0 or 16-16-16 at 15 g pot<sup>-1</sup> once a month and watered after each application. At flowering, provides 13-13-21 fertilizer at 15 g pot<sup>-1</sup> once a month. Foliar fertilizer, such as calcium, magnesium, boron, zinc and copper is also given, whenever the leaves are shown to be yellowing as due to micro elements deficiency. Applying high phosphorus and potassium fertilizers, such as, 8-16-24, 14-14-21 or 13-13-21 at 15 g pot<sup>-1</sup> once a month when new rhizome is developed. Plastic bag planting: the amount of fertilizer used for plastic bag planting should be less but more frequent than that for field planting, i.e. at 7-10 g bag<sup>-1</sup> for every three weeks (Department of Agriculture, 2006; Wichailak, 2006). H.M. the King's Initiative Centre for Flower and Fruit Propagation, Chiang Mai University, has developed a liquid fertilizer named "Banrai's Centre; BC-1", that is comprised of nitrogen (200 mg L<sup>-1</sup>), phosphorus (50 mg L<sup>-1</sup>) and potassium (200 mg L<sup>-1</sup>). This formula can

promote the number of plants per cluster and rhizome quality of *C. alismatifolia* Gagnep. (Ruamrungsri *et al.*, 2005).

### **2.4.3 Propagation**

*C. alismatifolia* Gagnep. propagation can be performed in several ways, these are:

#### **2.4.3.1 Seed propagation**

*C. alismatifolia* Gagnep.'s flowers prepare themselves for pollination once they start to bloom, 07:30 – 08:00 am until 10:00 am, about 2-3 months after the pollination when the pods are fully matured, the seeds should be quickly picked for the propagation. The collected seeds are planted in germination boxes containing sand mixing with rice husk charcoal at a 1:1 ratio. The seeds should be submerged in the 0.51-1.00 cm growing trays; when the seedlings shoot have approximately 5 leaves, they should be individually separated. Generally, the seedlings result from seed propagation require about 2 years to flower or yield the new rhizome.

#### **2.4.3.2 Rhizome division**

The rhizome division is an asexual propagation technique; a new plant gives rise to exactly the same characteristics as the mother plant. *C. alismatifolia* Gagnep. produces underground rhizomes. A single rhizome can produce up to 4-5 new curcuma plants. Towards the end of planting season, each plant has many connected rhizomes and could be separated as a single rhizome after they are dug up. The single rhizome then should be aerated to dry and mixed with the chemical fungicides to prevent it from fungus and should be stored in a well ventilated and shaded area.



### 2.4.3.3 Rhizome cutting

The rhizome is cut vertically at the center between two lateral buds. It is cut equally in half. Each piece of cut rhizome should consist of at least a perfect bud and a storage root. After that, the cut rhizome should be treated with a fungicide to prevent it from fungal infection through the wound. The cut rhizome should be planted promptly after treatment as it cannot be kept for a long period.

### 2.4.3.4 Tissue culture

A popular starting part for culturing *C. alismatifolia* Gagnep. by tissue culture method is the immature flower inflorescence that begins to emerge from pseudostem where it is still covered with bracts. Approximately 1.0 cm long of the inflorescence is excised and cultured in Murashige and Skoog (1962) cultured media adding BA and young coconut juice. The sprout from this culturing technique takes 2 years to flower and produce rhizome (Wannakrairoj, 1996).

### 2.4.4 Diseases and pest control

Bacterial pathogen is the most damaging agent causing wilt and rot diseases in *Curcuma*. *Ralstonia solanacearum* is a causal bacteria, the symptom appears at the lower leaves which will roll due to the lack of water and it is clearly prominent in the morning. The base of the plant and the new growth will appear succulent and leaf roll will spread to the upper parts, and then, the whole plant. The plant folds over, easily comes off the ground when pulled and finally, dries up or dies. The disease also causes rhizome and root parts to become succulent and transparent glassy appearance. It causes the rhizomes and roots to become dark and emit a rotting smell. The presence of nematodes will

exacerbate the problem (Department of Agriculture, 2006; Wichailak, 2006).

Nematode is an important factor in spreading the wilt disease and is commonly found in sandy soil. Control measure can be made by rotating crops that are not susceptible to the disease. Addition of organic matters, such as manure, fresh manure and humus, improves the physical properties of the soil, as well as, increase microorganisms that are antagonistic to nematodes. Collect nematode infected rhizomes and roots from the planting ground and dispose by burying or burning (Department of Agriculture, 2006 and Wichailak, 2006).

*Curcuma* is infested by very few insects except from the leaf roller. The grasshopper damages the leaves but can be controlled easily by synthetic pyrethroid, such as cypermethrin. Red spider mites cause spotting to the colorful bracts and can be controlled by dicofol with wetting agents.

## **2.5 Some factors affecting plant growth and development**

### **2.5.1 Light**

Light is required for photosynthesis and other growth activities. The light comprises tiny particles called photon that moves with high-speed ( $3 \times 10^8$  meter/second). The visible light is between 400 and 700 nanometers (4000 and 7000 angstroms) of wavelength. The light is an important energy source for photosynthesis. The plants have the reaction towards light which is influenced by light quality, light intensity and photoperiod. The energy in light is absorbed by chlorophyll of plants. The red and blue lights are more efficient spectrum for plant photosynthesis than the green light. The green

light is reflected from plants' leaves so the plants appear to be green to the eyes of the beholders (Hodgkiss, 2009).

There are 3 factors involve in the plants' photoperiodic response;

1) *Light intensity*

The light intensity depends on light source and distance between the plant and light. The light intensity affects plants' growth. The brighter light means more energy to the plants. Generally, mature vegetables and flower plants, which are grown under the sun light needs high light intensity (25 - 30 watts  $m^{-2}$ ) (a standard fluorescence gives 10 watts of light per the length of the bulb). Locating the light bulb 30 – 45 cm above the plants gives about 175 – 225 watts  $m^{-2}$  light intensity. The mature plants during flowering period need 8.976– 4.960 watts  $m^{-2}$  of light intensity and during leaves development need 1.496 – 8.976 watts  $m^{-2}$ , while flowering bulbs need 0.748 – 1.496 watts  $m^{-2}$  (Barkley, 2010; Vandre, 2006).

If the plants do not receive enough light, the stalks will begin to be withered and thin; the leaves are pale and the plants bend towards the light. It should be moved to the area near the light or extended the photoperiod. However, if the plants get too much light, the leaves and flowers will be pale white and become brown and eventually dried out; the size of leaves is smaller than usual and leaf margin is curly. The over exposing of light can restrict some kind of plants, such as a poinsettia, orchid and chrysanthemum from producing flower, Most plants generally need photoperiod in the range of 14 – 18 hours  $day^{-1}$ . The plants should be placed about 30 – 40 cm away from light (Vandre, 2006).

## 2) *Wavelength*

The wavelength that affects the growth of plants is between ultra-violet and infra-red of the light spectrum. The plants require more blue light (4,000 – 5,000 angstrom) than any other colors to stimulate chlorophyll production, encourage thick leaves, strong stems and compact vegetative growth. The pigments that correspond to the wavelength are such as carotenoids that has orange yellow color, absorbs blue light, that controls the leaves fall and fruits ripeness; riboflavin absorbs violet light and influences on photoperiodic responses of plant, plant's movement in result of phototropism, photosynthesis and controlling leaves-development. If the plants absorb only blue light, they become short in height, their internodes are short and the stalks are thick, the leaves are dark green and the flowers are suppressed. The red light (6,000 - 7,000 angstroms) controls the flower and seed production. The absorption of red light and far red light of the phytochrome affects photoperiodic responses which means that influences the seeds sprouting, roots development, rhizome production and plant's dormancy. The absorption only the red light gives tall and thin stalk plants. The yellow light has an influence on the chlorophyll production. Therefore, the plants need every color in the light spectrum to fulfill their development (Hodgkiss, 2009).

The light bulb radiates different wavelengths, such as tungsten incandescent light bulb produces long wavelength (red light) when the filament of the bulb is cool, however it gives short wavelength when the filament is hot. It emits heat energy with low luminous intensity so it is suitable in giving light for the plants that need low light intensity, however those plants may not produce flower. The incandescent light bulb

gives the red light, which has an effect on photoperiodic responses, and infrared light that becomes heat but has no influence on the growth of plants. If installing the light source too close to the plants, the leaves could be burned down due to much strong light intensity within narrow space can limit the light dispersion (Barkley, 2010).

The fluorescent light bulb is the popular light source as it is more efficient than incandescent light bulb. It also radiates more light than the later. It emits more red-blue light. There are many types of fluorescent light bulb, such as cool white fluorescent light bulb, of which it is the most efficient kind since it can be used with most plants and gives the light that is much appropriate for the growth of plants; warm white fluorescent light bulb and blue-red fluorescent light bulb have enough capability for certain kind of plants. This type of light bulb can also be designed to radiate red, green and yellow light or any specific light colors. It is also well designed for having more efficiency by radiating more of red and blue light and decreasing the radiation of green, yellow and orange light because the plants need some specific wavelengths in photosynthesis (Barkley, 2010).

### 3) Photoperiod

Photoperiod describes the duration of daylight in the daily cycle. Plants have the ability to measure photoperiod which then enables them to monitor seasonal changes, and to respond to it. The length of the day has an impact on plants' flowering, such as a long day plants, which need at least 12 hours of photoperiod per day for flowering and most of tropical plants need 12 – 16 hours of light to accomplish their growth. The photoperiod's control in a greenhouse is necessary for plants like *chrysanthemum*, *Kalanchoe Blossfeldiana* and *Euphorbia pulcherrima* that need short and long day to induce

flowering. There are 2 types of the control of photoperiod in greenhouse (Royal Horticultural Society, 2006; Vandre, 2006).

1. Artificial long day creation during short day (SD), the artificial long days are established by employing the incandescent, fluorescent or high-intensity discharge (HID) light bulbs. There are 3 techniques of giving artificial light, which are:

1.1 Day continuation lighting or day-extension lighting to 10.00 pm.

1.2 Pre-dawn lighting from 2 am to the sun-rise.

1.3 Night interruption lighting or night break lighting around 10.00 pm. - 02.00 am. This method gives less exposed light duration as compared to the 1.1 and 1.2 techniques with less energy cost.

Cyclic (intermittent) lighting in *chrysanthemum* is not necessary to continually give light from 10.00 pm to 02.00 am for long days. If using 20 foot-candles of light intensity from incandescent light bulb, it has been found that the plants required only 5 % of 30-minutes-long light exposure in order to achieve the long days. If applying 10 foot-candles of light intensity from incandescent light bulb, the plants needed simply 20 % of 30 minutes-long radiation. The percentage in giving light depends on light intensity the plants receive. Commercially, night-interruption lighting is always practicing during 10.00 pm - 02.00 am. The incandescent light bulb is usually used in cyclic lighting process. The cyclic lighting is generally allocated into 4 periods of turn on-off the light interval and each period, the light is turned on for 8 minutes (1<sup>st</sup> period: turn on the light at minute 1 - 8, 2<sup>nd</sup> period: turn off the light at minute 9 - 16, 3<sup>rd</sup> period: turn on the light

at minute 17 - 24 and 4<sup>th</sup> period: turn off the light at minute 25 - 32), this can save electrical cost by about 75 % (Barkley, 2010).

2. Artificial short day creation during the long day period (LD), the artificial short days can be accomplished by covering the plants with black clothe or opaque object. This helps to decrease light intensity. For efficient result, the light intensity should be decreased to 2 foot-candles or less. Usually, the plants should be covered around 04.00 – 05.00 pm. and stayed likewise for a night, then they would be uncovered around 07.00 – 08.00 am. This would give 8 hours of photoperiod for creating short days for the plants (Boyle, 2009).

#### **Light's influences on flowering**

The flowering of the plant is the progress of vegetative growth to reproductive growth. Flower is the reproductive organ of flowering plants. After the plant has attained its vegetative growth to its maturity, flower development may occur, where the leaf bud can be modified into a flower primordium. The changing at leaf bud is stimulated by environmental factors, such as long days and temperature; the mentioned factors might suit the development for the flower but probably not for the stalk. Additionally, the factors including humidity, nutrients and growth regulators are also important for flower initiation and development (Boonyakiat, 2007). The most important factor in flowering is light. The light has influences on the flowering in terms of photoperiod, wavelength and light intensity, or the combination of all 3 factors.

The plant's response to day length is divided into 3 major groups, which are:

1. Day-neutral plants – this kind of plants are able to flower in both long and short days, such as rose.

2. The plants that respond to long and short days – this group of plants can be separated into 2 subdivisions, which are:

2.1 Quantitative response (facultative response) is divided in 2 types, which are:

- Quantitative short-day plants
- Quantitative long-day plants

2.2 Qualitative response (obligate response) is sub-categorized into 2 types, which are:

- Short-day plant; the plant that flowers when day length is shorter than critical day length, such as chrysanthemum, *Kalanchoe Blossfeldiana*, and *Coleus Fredenici*
- Long-day plant; the plant, of which flowers when day length is longer than critical day length, such as *Calendula officinalis*

### 3. Dual day length requirement

The plant in this group is found to flower when the day length has to be varied in both short and long. It is found that floral initiation will not appear during the first period, however the second period of day length, the plant might produce flower bud. This group of plants do not have specific critical day length, but if at any period that the



flowers are produced fewest, that period could be critical day length. This group of plants can be divided into 2 sub-groups as the detail below:

3.1 Quantitative response is separated into 3 groups, which are:

- Intermediate-day plant – the plant's flowering is improved if the day length is not too short or too long.
- Short-long day plant – the short day length before long day length helps supporting flowering.
- Long-short day plant – under long day length and following by short day length the plant will be helped in flowering.

3.2 Qualitative response

- Intermediate-day plant – the flowering of the plant occurs under certain photoperiod; i.e., *Mikania scandens* flowers under 16 hours day length and 12.5 cycles.
- Short-long day plant – the plant is able to give flower under short day and then long day length, such as *Echeveria harmsii* flowers under 20 cycles of short day/ 10 cycles of long day.
- Long-short day plant – the plant yields flower under long day condition following by short day; i.e., *Bryophyllum daigremotianum* is treated under 60 cycles of long day/ 15 cycles of short day.

Naturally, short day plant doesn't flower during long day length circumstance, however if it is under short day for a definite time before the stage of development in long day length, the short day plant can also flower. In a similar extent, if

the long day plant is under the long day length for certain period before planting under short day length, this can result in the flowering of that plant as well. This flower inducing method is called photo-induction cycle (Boonyakiat, 2007).

Apart from critical day length that inducing the plant to flower, it also depends on cycle or number of day of photoperiod during critical day length. The lowest cycle or number of day that induces flowering in plant is called photo-inductive cycle, which is varied among the types of plant. Some kinds of plant might flower even if it is exposed to only one photo-inductive cycle. In some species of soybean, which are short day plants, need at least 2 days continually of photo-inductive cycle in order to flower. While winged bean requires 5 days of photo-inductive cycle for flowering. Radish is a long day plant and needs continually long photo-inductive cycle at 15 – 20 days for producing flower. The prolongation of photo-inductive cycle in some may plants enhance flowering like winged bean that needs 5 days of photo-inductive cycle to give 60 % of flower and if prolong photo-inductive cycle for 6-7 days, it will give 80 % of flower (Techapinyawat, 2005).

Age of plant affects flower induction. Mature plant can induce flowering. The 55-day-old winged bean could produce flower under photo-inductive cycle in 10 days, and the flowering would be increased if winged bean is older. However, if winged bean is younger than 50 days, photo-induction has no impact on plant's flowering. On the other hand, if the plant is very old, the quantity of the flower is increasing via photo-inductive cycle (Techapinyawat, 2005).

The long day plant requires shorter dark period than defined critical length in order to induce flower bud. The light-break given during the night shortens dark period and helps to induce flowering (Hopkins, 1999).

Wannakrairoj (1996) reported that the light-break given during the night for *C. alismatifolia* Gagnep. should begin on September 1<sup>st</sup> onwards in order to prevent the plant entering its dormancy. The leaf had to be exposed to the light for about 3 hours and that light was not necessary to be at high intensity. It needed 60 watts of light bulb hanging 50 cm above plant canopy. The light bulbs should be approximately 1.5 meters away from each other. This prevention from dormancy should also give enough humidity along with the light given.

Lepawit and Krasaechai (1995) researched on an effect of light intensity on long day induction in *C. alismatifolia* Gagnep. that planted during short day length (winter) by giving 6, 20 and 95 lux of light intensity from tungsten light bulbs and found that the light intensities of 20 lux and above given 4 hours day<sup>-1</sup> (07.00 pm – 10.00 pm) could prevent *C. alismatifolia* Gagnep. from dormancy during short day length. The stalk height, number of sprout, leaf and flower were greater than those receiving lower light intensity. The plants that received 6 lux of light intensity dry out about 30 percent.

In Taiwan, there was a study on the effect of a given light in association with covering the plot of *C. alismatifolia* Gagnep. with plastic sheet in order to maintain high temperature. *C. alismatifolia* Gagnep. entered dormancy period during October to November. Therefore, the light-break was given between 10.00 pm and 02.00 am and it was found that the given light could sustain flowering period to January 3<sup>rd</sup> and the

dormancy period was delayed to March 8<sup>th</sup>. Additionally this method improved quantity, quality, stem, floral diameter, floral color, and number of flowers. This suggested that the main factor causing *C. alismatifolia* Gagnep. to enter dormancy period was photoperiodism, while temperature was an associated factor (Chang, 2000).

### **2.5.2 Fertilizer**

Nitrogen, phosphorus, potassium and water are considered as the major limiting factors in crop growth, development and economic yield (Parry *et al.*, 2005). Although N, P and K frequently limit growth and development of several crop species under field conditions, the precise mechanisms by the limitation occurs are complex and variable depending on species, developmental stage and environment. Limited N, P and K supply decreases rate of cell division, cell expansion and cell permeability (Roggatz *et al.*, 1999), photosynthesis, leaf production, growth and yield. Hossain *et al.* (2010) found that kenaf plant growth, such as diameter, stem elongation and leaf number, were positively correlated with N, P and K levels until a certain level. In addition, N, P and K deficiency decreased plant height and photosynthesis of kenaf plants leading to lower biomass accumulation.

Nutrient limitation on net production had been reported by Shaver and Chapin (1980) they observed that nutrient limitation of growth and production was mediated by limitation of photosynthesis. Even the effect of fertilization on the photosynthetic rates and biomass accumulation in agricultural species is unclear. Nitrogen, Phosphorus and Potassium fertilization increase the growth rate of shoots of most vascular plant species, but depressed photosynthetic rates in all vascular species. Therefore, it appears that

nutrient limitation of growth is a direct limitation and not mediated through nutrient effect on carbon uptake rates and levels of available photosynthate. The reason for the reduction in photosynthetic rates with fertilization is not known. It is speculated that increases in growth with fertilization cause a dilution of other nutrients or factors, the effect of which is to depress photosynthesis. Moss photosynthesis is stimulated with fertilization. High NPK fertilizer levels stimulate photosynthesis more than low NPK fertilizer levels (Bigger and Oechel, 1982). Similar trend has been found in tea (*Camellia sinensis*), light saturation of photosynthesis occurred only at the higher fertilizer application rate but decreased in unfertilized tea or fertilized at the lower rate (Smith *et al.*, 1993).

Longstreth and Nobel (1980) reported that the net rate of CO<sub>2</sub> uptake for leaves of *Gossypium hirsutum* L. was reduced when the plants were grown at low concentration of NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>2-</sup>, or K<sup>+</sup>. In addition, the water vapor conductance was relatively constant for all nutrient levels, indicating little effect on stomatal response. Although leaves under nutrients stress tended to be lower in chlorophyll and thinner, the ratio of mesophyll surface area to leaf area did not change appreciably. Thus, the reduction in CO<sub>2</sub> uptake rate at low nutrient levels was due to a decrease in the CO<sub>2</sub> conductance.

## 2.6 Photosynthesis

Photosynthesis is a physiological system that plant absorbs light energy and then turns it into chemical energy. Chemical energy is later accumulated in carbohydrate compound; inherently contains high energy, which are sugar and starch. These components

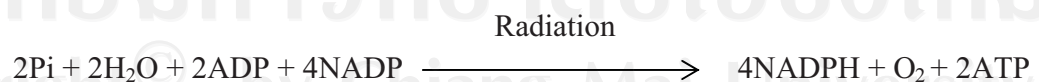
feed plants via respiration system (Techapinyawat, 2005). The photosynthesis is categorized as a reduction process of carbon dioxide, so the photosynthesis measurement can be made by measuring from an exchanging of carbon dioxide between the air and plant leaf.

The plant's photosynthesis consists of 3 processes:

1. Carbon dioxide diffusion;  $Pn$  - it is a diffusion process of carbon dioxide from the air around leaf surface into photosynthesis center. The diffusion rate of carbon dioxide relies on the difference of carbon dioxide concentration in the air and the chloroplast, as well as other resistances that will occur during the diffusion of carbon dioxide. These resistances concerned are: air resistance ( $R_a$ ), stomatal resistance ( $R_s$ ), and meshopyll resistance ( $R_m$ ). The relationship of carbon dioxide diffusion ( $Pn$ ) and the parameters involved can be concluded as the following equation: (Sampet, 1999)

$$Pn = \frac{[CO_2]_{\text{air}} - [CO_2]_{\text{Chloroplast}}}{R_a + R_s + R_m}$$

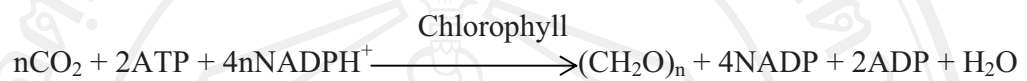
2. Light reaction – this is a changing of solar radiation to plant's biochemical compounds. In this process, the water oxidation reaction also occurs; the final results from the light reaction are therefore biochemical energy and oxygen.



The solar radiation energy is a major factor for this process, so it is always called light reaction. The light reaction occurs in the thylakoid of chloroplast. This process uses the energy from solar radiation for hydrolysis called photolysis or photo-oxidation to yield hydrogen, ion, electron and oxygen. Besides, the resulting are products

from this process also ATP and NADPH chemical energy, which will be utilized in the third mechanism of biochemical reactions (Techapinyawat, 2005).

3. Biochemical process or CO<sub>2</sub> fixation – this is a process that Rubisco enzyme (ribulose-1,5-biphosphate carboxylase-oxygenase) reduces carbon dioxide to a sugar form or carbohydrate by using ATP and NADPH energy as detail below:



The light is not required for this process, so it is normally called dark reaction. The dark reaction will synthesize sugar from carbon dioxide without using light energy directly but the consequence from light reaction, which is the biochemical energy in a form of NADPH and ATP. This dark reaction occurs in the stroma of chloroplast and it has different procedures of the CO<sub>2</sub> fixation pattern, which in turn, the plants can be grouped into C<sub>3</sub>, C<sub>4</sub> and CAM plants accordingly.

The C<sub>3</sub> plant is the plant that fixes carbon dioxide in the air and combines with ribulose-1,5-bis-phosphate (RuBP) by using RuBP carboxylase enzyme and decomposing it to produce 3-phosphoglyceric acid (3-PGA), which is a 3-atom carbon substance; then the 3-PGA will later change into other chemical compounds and eventually becomes ribulose-1, 5-bis-phosphate. This group of plant is such as soybean, tobacco and most of plants in temperate zone.

The C<sub>4</sub> plant is mostly the tropical plants. This type of plant has Kranz anatomy physical structure, which means that it contains bundle sheath cells (cells which circle around the vascular bundle) that also accommodate chlorophylls. Therefore, this kind of plant has twice CO<sub>2</sub> fixation: firstly, it occurs in the mesophyll of leaf as carbon dioxide

will react with phosphoenol pyruvate (PEP) by relying on PEP carboxylase enzyme's activity; the result is chemical compound with 4-atom carbon (e.g. malate, aspartate and oxaloacetate). The plant, therefore, is called C<sub>4</sub>. These chemical compounds will be delivered to cell in the bundle sheath and converted to pyruvate and CO<sub>2</sub> in order to be employed in Calvin cycle later in the process. The plants in this group are sugar cane, corn, millet and tropical-grass-family plants.

The plant in CAM group (Crassulacean acid metabolism-pathway) is the plant that can grow in dry area, such as desert plants and succulent plants. This plant possesses CO<sub>2</sub> fixation process to build carbon compounds in a form of organic acids during the night, such as malic acid. While during the day, it will release the fixed carbon dioxide to Calvin cycle in the mesophyll cell in order to create sugar.

In general, the plant's photosynthesis process can be concluded as the following chemical equation:



Practically, photosynthetic rate measurement can be made by measuring carbon dioxide exchange rate; CER or CO<sub>2</sub> assimilation rate instead of calling it as the photosynthesis rate (Chuennakorn and Yingjajaval, 2007; Techapinyawat, 2005; Hall *et al.*, 1993).



### 2.6.1 Influential factors on photosynthesis

There are many factors influence on photosynthesis capability of plants, and these can be separated into the external and internal factors. For external factors, they are light intensity, temperature and relative humidity of the surrounding environment. The internal factors are leaf life and the amount of chlorophylls in leaf.

Likewise, these factors can be divided into 2 groups; i.e. internal and external factors.

#### 2.6.1.1 Internal factors

These mean the type of plant, physical structure of plant, genetic conditions, age or life cycle of plant, (Techapinyawat, 2005) and the leaf age that are relevant to capable of photosynthesizing, however they also depends on environment (Jones and Lazenby, 1988). Kasemsap (2006) and Techapinyawat (2005) found that not only too old leaf but also too young leaf had low ability to perform photosynthesis, which inevitably affected on plant growth. Kasemsap *et al.* (1993) studied the relationship between leaf position and total photosynthesis ( $P_{max}$ ) in leaves of 3 different species. The result revealed that the  $P_{max}$  decreased with increasing leaf position. The average  $P_{max}$  values of the leaf positions at 8, 10 and 12 were significantly different. Besides, an increase in leaf number and leaf surface area could increase photosynthesis (Kasemsap *et al.*, 1993). However, excess amount of leaves caused shading to other leaves, thus total photosynthesis would decrease. For example, *Dendrobium* sp. the tree character exhibited to be a large bush. Leaves at low level of the canopy obtained less light intensity, hence the total photosynthesis was less than the upper part that was fully exposed to sun light (Chuennakorn and Yingjajaval, 2007).

### 2.6.1.2 External factors

#### *Light*

The light is an energy source of plant for photosynthesis. From a previous study, it was reported that the light used in photosynthesizing process was only at the average of 1 - 5 % of all the light that radiated upon the plant, or only 3 - 10 % for a proper leaf area index (Sampet, 1999).

The reaction of CO<sub>2</sub> assimilation rate is called light response curve. The relationship between CO<sub>2</sub> assimilation rate ( $A$ ) and quanta ( $Q$ ) is curvilinear in pattern that comprises different periods as follows. In the dark condition, there is only respiration, the  $A$  value will be minus; but when  $Q$  increases in its value,  $A$  gradually increases the value to 0, which is so-called the light compensation point. However, before reaching this point, it will possibly be Kok effect as a result of the fast increasing of  $A$  when  $Q$  rises. And if exceeding this point,  $A$  will relate to  $Q$  in a linear pattern if  $Q$  equals to 50 - 200  $\mu\text{molm}^{-2}\text{s}^{-1}$ . The slope value of the relationship between  $A$  and  $Q$  is the quantum yield ( $\Phi$ ) of photosynthesis. If  $Q$  exceeds this point, the reaction pattern of  $A$  will be convexity until reaching  $A$  saturated point where the light at this point is called the light saturated point, while the photosynthesis rate is called  $A_{sat}$ . The quantum yield means quantity of mole of fixed carbon dioxide by plant leaf per one mole of light upon the plant leaf (Hall *et al.*, 1993), which indicates the efficiency of light used in that plant.

#### *Temperature*

In dark reaction, photosynthesis process depends on enzyme's performance, of which always highly sensitive to temperature changing. Generally, each kind of plant has

different suitable temperature ranges for photosynthesis from 5 to 40 °C. During high temperature condition, the C<sub>4</sub> plant retains its photosynthesis better than the C<sub>3</sub> plant (Techapinyawat, 2005). The C<sub>3</sub> plant performs the highest photosynthesis rate within the temperature range of 10 to 25 °C, whereas, the C<sub>4</sub> plant achieves the highest photosynthesis rate at 30 – 40 °C (Sampet, 1999). Hew *et al.* (1969) studied the effect of temperature on photosynthesis and found that apparent photosynthesis decreased with increasing temperature, from 20 to 40 °C in sunflower, soybean, watermelon, eggplant and jackbean. In leaf of dicotyledonous plants, it appeared to be the decrease in apparent photosynthesis between 20 to 30 °C. Moreover, temperature also plays an important role in plant respiration rate. The plant respiration rate will be higher when temperature is high, so if the temperature is higher than proper temperature range for photosynthesis, the light will cause the respiration rate to become higher than photosynthesis rate, and this leads to the reduction in the net photosynthesis (Sampet, 1999). Temperature is the primary factor that affects ginger's sprouting and growth, and it is commonly used to hasten or delay its development (Paz *et al.*, 2005).

It can be seen that light intensity and temperature are the factors that govern the photosynthesis rate and effectiveness of photosynthesis process in plant. Besides, each kind of plant holds different appropriate temperature range and light intensity for most effective photosynthesis. The plants in temperate zone have less suitable level of the concerned factors than those in the tropical zone. Though the low light intensity always restricts photosynthesis rate of most plants, and if the plants are under photoperiod of strong light intensity for long time, the photosynthesis is possible to cease as well

(Techapinyawat, 2005). This happens because the plants are entered into the photo-inhibition state; i.e. too strong light intensity may cause malfunction in pigment for photosynthesizing process and affect the central system of the photosynthesis (Krause and Weis, 1991).

Thomas and Vince-Prue (1997) reviewed that red light was more effective than other wavelength regions, such as blue, green or far-red light in night break treatment of potatoes and begonia. Giving a red light pulse in the middle of dark period to plants grown in short day led to inhibition of flowering in short day plant, but promotion in long day plant, inhibition of tuberization. Low light integrals had a retarding effect on flower growth resulting in smaller flowers. Carvalho *et al.* (2002) found that light conditions influenced flower bud removal, flower size and number of cut chrysanthemum. Light quality has been shown to play an important role in morphogenesis and photosynthesis (Kim *et al.*, 2004).

Plant growth and bioproductivity are ultimately dependent on leaf photosynthesis. Prolonged changes in photosynthesis photon flux density (PPFD) alter the anatomical, physiological, and biochemical properties of the leaf. Plant grown under reduced radiation fluxes was found to have reduced carbon exchange rate, low stomatal conductance, and reduced mesophyll area, resulting in slower photosynthetic rate (Gutiérrez and Meinzer, 1994).

#### *Carbon dioxide*

In photosynthetic process, carbon dioxide in the air diffuses into the plant via stoma, then absorbs through a gap between stomatal cells into the cell wall of mesophyll,

cytoplasm and chloroplast. This diffusion rate depends on the difference of carbon dioxide's intensity in the air and the plant cell. The ability of chloroplast to decrease the intensity of carbon dioxide in the leaf or to use carbon dioxide for photosynthesis process can be measured by determining the CO<sub>2</sub> compensation point, which means an equilibrium when carbon dioxide's quantity that the plant releases by respiration equals to the quantity that diffuses into the plant for photosynthesis (Sampet, 1999).

The relationship between photosynthetic rate ( $A$ ) and carbon dioxide intensity in the plant's stoma (internal CO<sub>2</sub>;  $C_i$ ) has the hyperbolic or asymptotic pattern of reaction. From  $A/C_i$  plot, it will consist of an initial linear response for carbon dioxide fixation. The initial slope value of  $A/C_i$  gives the carboxylation efficiency or capability of the Rubisco enzyme. Furthermore, the  $A/C_i$  also explains that  $A$  might be limited by quantity and efficiency of Rubisco in carboxylation process. At the end of  $A/C_i$  provides the ability of leaf in creating RuBP<sub>ase</sub> enzyme for the process of carbon dioxide fixation (Hall *et al.*, 1993).

#### *Humidity and water quantity*

Water is an important reactant for photosynthesis, but the plant utilizes only about 0.1 % of total root absorbed water in photosynthesis. Water is a source for electrons employed in the photosynthesis. If the plant lacks of water, a stomata will close, therefore carbon dioxide will not be supplied for photosynthesis and thus, the photosynthesis will decrease. During flood, the soil is soaked with water; this makes the root lacks of oxygen for respiration, so the plant is deprived of energy to absorb water and hence affects the photosynthesis rate as well (Techapinyawat, 2005).

Water level within the plant is important to the control of plant's stomata. The stomata of the plant has a role in various processes of metabolism, such as photosynthesis and respiration. If the stomata of plant is closed decreasing the transpiration during water deficiency, the photosynthesis rate and respiration will be decreased as well. Therefore, the respiration, photosynthesis and transpiration processes are closely related and cannot be separated.

#### *Chlorophyll fluorescence*

A light absorbing of a molecule in any substance depends on energy level or wavelength of each kind of light. The visual light spectrums are ranged approximately 380 - 760 nanometers of wavelength. When a molecule of substance absorbs this wavelength, it will change molecule's energy level, which will cause it to be in an excited state of electron. At normal stage before molecule absorbing light energy, a molecule is in its ground state. The higher energy of electron or molecule from normal state and being in the excited state is called excitation energy, which is an unstable energy and occurs in very short state within a second. To make use of this energy, it has to continually transfer electron or energy via adjacent molecule of pigment through the photosynthetic reaction center. If this excitation energy cannot transfer electron to other substance within  $10^{-9}$  seconds, the excitation energy will be transformed into heat or changed to be light with longer wavelength or fluorescence (Techapinyawat, 2005).

For most plant, the energy will be absorbed by the molecule of chlorophyll in order to be used for photosynthesis. However there will be some energy that is not brought into use in this reaction, the plant, therefore, has its mechanism in releasing this

energy in several forms, such as by reflection of radioactive and emission of fluorescence (Hall *et al.*, 1993).

In normal state, when the plant leaf is under dark for about 30 minutes, it has been found that the electron acceptor in Photosystem II (PSII) will be at the ground state and is able to open to receive maximum energy from solar radiation. Nevertheless under this state, chlorophyll is also able to radiate minimum fluorescence, so-called  $F_o$ . The plant immediately changes it to be in excited state when receiving electron acceptor and the minimum fluorescence ( $F_o$ ) from chlorophyll, and that will increase to maximum fluorescence ( $F_m$ ), then this value will drop down to  $F_o$  level again when the energy is transferred to Photosystem I (PSI), so the electron acceptor will receive energy from solar radiation again. The phenomenon of the change in the chlorophyll fluorescence value is called Kautsky curve. The difference between  $F_m$  and  $F_o$  value is called variable fluorescence ( $F_v$ ). The ratio of  $F_v/F_m$  gives the capability of PSII in plant's chlorophyll to acquire energy from solar radiation and transfer to PSI; this value has a relationship with an efficiency of chemical reaction of light using in photosynthesis, which is called quantum yield (Björkman and Demmig, 1978).

Chlorophyll measurement is, therefore, one of the methods that can indicate an efficiency of photosynthesis. Nowadays, the chlorophyll measurement technique has been improved by an invention of an equipment to study the reaction of plant physiology to stressful environment (Flagella *et al.*, 1994). If applying this technique to computer operation, the measurement will be simple and fast and the plant won't be disturbed

(Selmani and Wassom, 1993). Additionally, it can be used either for fieldwork or in a research lab (Olaf and Snel, 1990).

Flagella *et al.* (1994) studied chlorophyll fluorescence in wheat, which was a C<sub>4</sub> plant; found that wheat developed in high water condition would give higher  $Fv/Fm$  and yield than wheat grown in low water condition. Similarly, Selmi and Wassom (1993) reported that in an experiment of chlorophyll fluorescence measurement of wheat in high water condition, the  $Fv/Fm$  was higher than that in low water condition.

Hidekaza *et al.* (1994) studied chlorophyll fluorescence in cucumber leaf and found that  $Fv/Fm$  had a linear relationship with the quantum yield of photosynthesis, which implied that chlorophyll fluorescence value could be used to explain the efficiency in plant's photosynthesis.