Chapter 2

Review of literature

2.1 Origin and distribution of *Curcuma*

The genus *Curcuma* L. belongs to the family Zingiberaceae which is composed of about 70 – 80 species of rhizomatous annual or perennial herbaceous (Purseglove, 1974). The genus has been divided into two subgenera of *Eucurcuma* and *Paracurcuma* by several taxonomists using different morphological traits. The family Zingiberaceae commonly known as ginger family is a unique plant family comprising perennial aromatic forest plants. It is one of the economically important flowering plant families of the tropics that yields spice, dyes, perfumes, medicines and ornamental flowers (Haywood, 1985).

The species belonging to the 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 ranged 20 to 30 °C. A rainfall of 150 cm or more or an equivalent amount of irrigation is essential for optimum growth and development of *Curcuma* species. Ideal soil requirements for *Curcuma* growing are loose, friable loamy or alluvial suitable for irrigation that should have efficient drainage capacity. The species are naturally found in mixed deciduous tropical forests and tropical broad-leaved evergreen forests of the tropical and subtropical regions. The geographic distribution of the genus reaches from India to Thailand, Indochina, Malaysia, Indonesia and finally to northern Australia (Apavatjrut *et al*., 1999). Along with ginger, *C. longa* is

probably taken from India, south-east Asia, China and northern Australia to the West Indies and South America by the Spaniards (Apavatjrut *et al*., 1999; Cao and Komatsu, 2003; Joe *et al*., 2004; Maciel and Criley, 2003; Majeed *et al*., 1995).

2.2 Morphology and taxonomy of *Curcuma alismatifolia* Gagnep.

Curcuma alismatifolia is one of high potential plants in the world market. It is in a subgenera *Paracurcuma*. The rhizome is formed from lateral buds at the base of the pseudostem when the plant is mature. Because its growth is more horizontal than vertical, thus it is called a tuberous rhizome, it is attached with 2 to 3 stolon-like storage organs termed storage roots (Phongpreecha, 1997).

The storage roots are thought to act as storage organs and play an important role in growth and development (Hagiladi *et al*., 1997b). The number of storage roots is also thought to affect flowering time, inflorescence stem length and number of stems per rhizome (Phongpreecha, 1997). If rhizomes are stored for a long period of time, the storage roots will gradually dry out and the rhizome will be the last organ to dry. Thus, the more storage roots on a rhizome the longer it can be stored (Phongpreecha, 1997).

Foliage appears after the rhizome dormancy has broken. Each shoot composes of a psudostem and leaves. The stem is made up of an axis covered by overlapping sheathing leaf. Leaves are oppositely arranged in a flat, two dimentional plane (Phongpreecha, 1997).

Inflorescence bears prominent spiral bracts, which are laterally fused to form pouches. Each pouch subtends a cincinnus of two to ten flowers that contains a single

versatile anther. The terminal bracts form a sterile cluster called a ‘coma’, very long and often brightly coloured (Sirirugsa *et al*., 2007).

2.3 Physiology of flowering

The general body plan of plants is established during embryogenesis, when the undifferentiated meristematic regions of root and shoot are set aside. However, much of plant development occurs postembryonically, through the reiterative production of organ primordia at the shoot apical meristem (SAM). In most species, the SAM initially gives rise to vegetative organs, such as leaves, but at some point the SAM makes the transition to reproductive development and the production of flowers.

This change in the developmental phase of primordia initiated at the SAM is controlled by environmental and endogenous signals. However, unlike many developmental transitions in animals, the SAM of plants is not irreversibly "committed" to reproductive development once flowering commences. In some species and genotypes under certain environmental conditions, leafy shoots are formed after flowers in a phenomenon known as inflorescence reversion. This observation implies that the genes and processes involved in the transition to flowering are required both to initiate and maintain reproductive development (Levy and Dean, 1998)**.**

2.3.1 Model of flowering

Over the years, physiological studies have led to three models for the control of flowering time (Bernier, 1988). The florigen concept was based on the transmissibility of substances or signals across grafts between reproductive "donor"

shoots and vegetative "recipients." It was proposed that florigen, a flower-promoting hormone, was produced in leaves under favorable photoperiods and transported to the shoot apex in the phloem. The identification of a graft-transmissible floral inhibitor also led to the concept of a competing "antiflorigen." Many research years were consumed in hunting for florigen in the phloem sap, but its chemical nature has remained elusive (Evans, 1971).

The inability to separate the hypothetical flowering hormones from assimilates led to a second model, the nutrient diversion hypothesis. This model proposed that inductive treatments result in an increase in the amount of assimilates moving to the apical meristem, which in turn induces flowering (Bernier, 1988).

The assimilates are the only important component in directing the transition to flowering was superseded by the multifactorial control model, which proposed that a number of promoters and inhibitors, including phytohormones and assimilates, are involved in controlling the developmental transition. According to this model, flowering can occur only when the limiting factors are present at the apex in the appropriate concentrations and at the right time. This model attempts to account for the diversity of flowering responses by proposing that different factors could be limiting for flowering in different genetic backgrounds and/or under particular environmental conditions (Levy and Dean, 1998).

2.3.1.1 The flowering hormone or florigen/antiflorigen model

Florigen was first described by Russian plant physiologist Mikhail Chailakhyan in 1937, who demonstrated that floral induction can be transmitted through a graft from an induced plant to one that has not been induced to flower

(Bernier, 1988). Lang (1952) showed that several long-day plants and biennials could be made to flower by treatment with gibberellin, when grown under a non-inducing photoperiod. This led Chailakhyan to modify his florigen hypothesis to postulate two classes of flowering hormones: Gibberellins and Anthesin.

Chailakhyan postulated that during non-inducing photoperiods, long-day plants produce anthesin, but no gibberellin while short-day plants produce gibberellin, but no anthesin. However, these assumptions did not account for the fact that short-day plants grown under non-inducing conditions (thus producing gibberellin) would not cause flowering of grafted long-day plants that were also under non-inductive conditions (thus producing anthesin). The flowering hormone has eluded scientists for over sixty years, as the flowering response has been found to be increasingly complex. A possible hypothesis is that florigen does not exist; rather, a particular ratio of other hormones must be achieved for the plant to flower. However, recent experiments suggest that florigen does exist. Its existence is substantiated by the substance that triggers blooming is produced or activated in the leaves of the plant, and must be given time to pass out of the leaves before the plant can flower (Bergfeld *et al*., 2003).

No other botanical substance has so long and so unsuccessfully been searched for as for the flowering hormone or florigen. It did at least became a name, and its existence could be proven by grafting experiments. Phytochrome that is localized within the leaves is required for the control of flower formation (Bergfeld *et al*., 2003). The conditioning, i.e. the signal necessary for stimulation of flowering or for suppression of flower formation is also generated within the leaves. The majority of the classic grafting experiments have been performed with *Nicotiana*-

species. *Nicotiana sylvestris* is a long-day plant and *Nicotiana tabacum,* var. Maryland Mammoth (M.M.) is a short-day plant. Most other *Nicotiana tabacum*-species are day-neutral.

If *Nicotiana sylvestris,* for example, is cultivated under short-day conditions, it does not develop flowers, but if a leaf of a *Nicotiana tabacum,* M.M. plant that has been cultivated under short-day conditions is grafted to *Nicotiana sylvestris,* then it is stimulated to flower. This means that the *Nicotiana tabacum,* M.M.-leaf has produced a substance that is transferred to the recipient after grafting and that causing flower formation. This has proved the existence of a florigen (Bergfeld *et al*., 2003).

2.3.1.2 Nutrient diversion hypothesis resource allocation

Nutrient content at the SAM changes at the time of floral transition, induced by change in transport of sucrose and other sugars to the apex. The nutrient diversion theory of floral transition is based on changes in sucrose concentration during floral transition, with higher sucrose levels at the SAM following floral inductive treatments in species like *Sinapis alba*. Changes in the relative ratio of sugar and nitrogen transport to the apex is also implicated in the nutrient diversion theory. No genetic evidence for this model exists to date, but analysis of carbohydrate to nitrogen ratios prior to and during floral transition show that under vegetative condition, the ratio is consistent but at time of flowering nitrogen transport becomes proportionally greater to the apex. However, more recent work has shown that increase in sucrose level at the apex changed at the time of flowering in *Fuchsia hybida* regardless of whether or not an inductive photoperiod occurred. It is possible that changes in sucrose concentrations, or other nutrients, is a result of floral transition and not the signal that

induces flowering. Indeed, when long day inductive signals at low light levels are applied, the amount of sucrose at the apex does not increase, while plants still flower in response to the day length changes. This suggests that sucrose marks photosynthesic activity, and that the photoinduction pathway does not simply require photosynthesis to carry out induction, while these results indicate that nutrient changes at the apex are not required for floral evocation, and thus, it is still not known if these changes can induce flowering (Charles, 2006).

2.3.1.3 Multifactorial control model

Flowering is a unique and integrated process of very complex nature and multifactorial control, that has been studied extensively, from ecophysiology to biophysics aspects (Bernier *et al*., 1981a,b; Bernier *et al*., 1993; Kinet *et al*., 1981; Kinet, 1993). Most of the plants react to environmental signals regulating the transition into flowering, since all individuals of a given species have to bloom synchronously for the success of crossings and they also should complete sexual reproduction under favorable external conditions (Bernier *et al*., 1993). In general, natural flowering is stimulated by regular seasonal changes of climatic conditions, such as photoperiodism, thermoperiodism and water balance. Such changes are sensed by different organs of the plant, the photoperiod by the leaves; the temperature by all parts of the plant, although low temperatures are preferably sensed by the stem apex; and the water deficit by the roots (Bernier *et al*., 1993). The presence of at least one leaf on the plant is necessary for the perception of photoperiodic stimuli (Wareing and Phillips, 1981; Bernier *et al*., 1988).

According to O'Neil (1992), any explanation about the control mechanisms of flowering by photoperiod should consider the presence not only of promoters but also of inhibitors, which is in agreement with the model of control of the "evocation" proposed by Bernier *et al*. (1981b). According to these authors, the factors are not the same for different species, and can be synthesized in leaves, roots, stem apex and other organs. If just one factor is absent, the process will not continue, but generally all of them are present under inductive conditions. Some evidences show that flowering in the meristem may consist of several phases, each one being activated individually (Bernier *et a*l., 1992). However, numerous questions still need to be clarified.

2.3.2 Flowering process

The transition from the adult vegetative phase to the reproductive phase (the transition to flowering) involves major changes in cell differentiation and morphogenesis at the shoot apical meristem (SAM). The SAM consists of a population of pluripotent stem cells that are formed during embryogenesis, and give rise to all shoot structures (Bowman and Eshed, 2000). The SAM, which is located at the tip of the shoot apex, is responsible for the production of lateral organs and stem tissues. SAMs are dome shaped and can be subdivided into two main regions, i.e. the tunica and the corpus. The tunica consists of the epidermis (L1) and the underlying cell layer (L2), both of which undergo cell divisions in an anticlinal plane. In contrast, the cells below the L2 represent a mass of cells called the corpus (L3), which divides in all directions. An alternative and much used classification of meristemic areas is to divide them into cytological zonations, a peripheral zone, a central zone

and a rib zone. Lateral organs derive from the peripheral zone, stem cells derive from the rib zone and the central zone consists of cells that replenish both the peripheral and the rib zones (Steeves and Sussex, 1989; Sussex, 1989; Meyerowitz, 1997; Barton, 1998).

After the transition to flowering the SAM becomes an inflorescence meristem, which can be distinguished from vegetative SAMs early in their development by their larger size and different shape. During the floral transition there is a marked increase in cell division in the central zone. The first primordia are produced by the primary inflorescence meristem of plant form cauline leaves, and the secondary inflorescences are produced by the associated meristems. Subsequently floral primordia are produced in the peripheral zone of the inflorescence meristem (Weigel and Jürgens, 2002). A similar situation may prevail in *Arabidopsis thaliana*. Besnard-Wibaut (1970, 1977) noted the increased percentages of nuclei incorporating labelled thymidine (presumably indicative of increased mitotic frequency) in several zones of the *Arabidopsis* SAM in response to floral induction. On the other hand, Vaughan (1955), Miksche and Brown (1965) and Hempel and Feldman (1994) reported SAM enlargement and mounding-up before initiation of the first floral meristem, as well as elongation of rib meristem cells causing the elongation of the stem axis.

Apical meristem developmental stages

In the *Glomerulate* inflorescence, apical meristem development in this type of inflorescence progresses up to the formation of a terminal flower. In both apical and axillary buds, differentiation progresses basipetally. Axillary buds grow

giving origin to the second order axes, and an hermaphrodite flower is formed at the apical meristem of these ramifications. From these second-order axes, the third-order axes are formed. Flower-bearing lomeruli are supported by these third-order axes. The main stages of the apical meristem development observed in the *Glomerulate* inflorescence are as follows.

G0. Vegetative: in this stage, the apical dome is hemispherical, and appears fully covered by leaf primordia. These primordia hide subjacent bud primordia.

G1. Early reproductive: the first sign of the transition towards flowering is an increase in the rate of growth of the apical meristem with respect to leaf primordia that leads to the emergence of the apical dome from among the leaf primordia.

G2. Exposed apex: the rate of axillary bud growth increases in relation to that of leaf primordia and thus become visible.

G3. Beginning of the differentiation of the terminal flower: the apex assumes a pentagonal shape owing to the appearance of five sepal primordia.

G4. Beginning of gynoecium differentiation: the apex expands to form a globose body, around which a rim soon develops (the future ovary wall) encircling a small dome (the future solitary ovule). The sepal and stamen primordia are now clearly distinguished around the ovary.

G5. Ovary wall partially covering the ovular primordium, which in fresh material is distinguished by a more intense green colour. The two thecae of each stamen are clearly distinguishable.

G6. Ovary wall almost fully developed, ovular primordium no longer visible.

G7. Onset of differentiation of stigmatic branches. Four primordial stigmatic branches are apparent at this stage and two pollen sacs are already differentiated in each theca. After the differentiation of stigmatic branches there follows a period of growth in size, elongation of stigma and stamen pedicel, in parallel with pollen and ovule formation and maturation.

G8. Anthesis: defined as the time of the presentation of the anthers in perfect flowers and the elongation and emergence of the pistil in pistillate flowers. Pistillate flowers are characterized by three very large stigmatic branches (one stigmatic branch is aborted) and no functional anthers (Bertero *et al*., 1996).

2.3.3 Factors affecting flowering

The central subject of the floral initiation physiology consists of understanding which factors act in the transformation of the stem apex into a floral primordium and how they play their roles (Lang, 1952). The knowledge of those signals has a fundamental and practical importance for a more rational crop exploration. According to Bernier (1988), the main environmental factors responsible for floral induction are the photoperiod (daylength - hours of light) and temperature (vernalization - cold effect). Yet according to this author, it is required that the plant reaches an adequate developmental stage to be induced to flower, being necessary that the leaves capture photoperiodic signals. Once totally accomplished the differentiation of the caulinar meristem into floral primordium, the latter becomes unable to retake the vegetative growth. That is why vegetative growth and reproductive development in plants are events considered mutually exclusive.

2.3.3.1. Genetics factor

Defining which factors affect flowering is important for a better understanding of plant growth and development and offers an opportunity to study the interactions of environmental cues, chemical signals, and gene expression.

Recent advances in genetics have made it possible to study the genetic control of flowering. In the model plant *Arabidopsis thaliana*, many genes affecting flowering time or the transition from the vegetative to the reproductive phase have been identified and a number of them have been cloned (Wilfred *et al*., 2002). This has resulted in a model in which meristem identity genes activate a developmental program that enables the shoot apical meristem to produce reproductive structures. The meristem identity genes can be activated via three different pathways. One pathway involves a set of autonomous genes (which are turned on at a specific developmental stage), the second pathway involves genes that are responsive to the photoperiod, and the third pathway includes genes that respond to the phytohormone gibberellins (Wilfred *et al*., 2002).

Orthologs of *Arabidopsis* flowering genes have been identified in several other species, including pea, snapdragon, rice, maize, pine and ryegrass (Wilfred *et al*., 2002). In addition, transformation of one plant species with constructs will result in the over-expression of flowering genes from another plant species imposing their effects on flowering of transferred plant. This indicates that the function of several flowering genes is conserved between plant species. On the other hand, there is evidence that the transition to flowering is not governed by a universal set of signals. Many plant species, including maize and other grasses are much less dependent on the photoperiod and gibberellin than *Arabidopsis*, suggesting that

different signals may be required for flower development. This is conceptualized in the "multifactorial control" hypothesis in which a combination of chemicals, including phytohormones, assimilates and minerals interact with genetic components in the process of floral initiation. The recent cloning of the *Indeterminate (Id1)* gene from maize also illustrated incomplete understanding of the floral transition process. The *Id1* gene is expressed in developing leaves prior to their transition from sink to source tissue. Its sequence shows similarity to a transcriptional regulator, and the gene product may play a role in the movement of a flowering signal in developing leaves. The *Id1* gene is different from any other of the flowering genes isolated from *Arabidopsis* so far (Wilfred *et al*., 2002).

2.3.3.2. Size of storage organ

An important factor for geophytes in determining whether or not flowering will occur in relation to the size of the storage organ (Rees, 1992). The critical size is genus or species dependent. For *C. alismatifolia*, rhizomes with greater number of storage roots were found to sprout faster than those with fewer number of storage roots (Phongpreecha, 1997). Hagiladi *et al*., (1997b) found that the presence of storage roots markedly accelerated flowering in *C. alismatifolia*. However, they found no significant difference in flowering time between plants with different number (greater than 2) storage roots. They also found that plant originating from rhizomes without storage roots did flower, bud very late. Emergence, flower initiation, and flowering time of rhizomes with more storage roots were earlier than those with fewer storage roots (Phongpreecha, 1997). The greater the number of storage roots translated to higher yield of inflorescences (Hagiladi *et al*., 1997b).

2.3.3.3 Hormone

2.3.3.3.1 Cytokinins (CKs)

Cytokinins were discovered during the 1950s because of their ability to induce plant cell division (Miller *et al*., 1955). Shortly after their discovery, Skoog and Miller, (1957) coined the auxin-cytokinin hypothesis of plant morphogenesis. The hypothesis predicted that cytokinin, together with auxin, plays an essential role in plant morphogenesis, having a profound influence on the formation of roots and shoots and their relative growth.

The well-documented stimulatory effect of added cytokinins on growth and differentiation of cultured plant cells, flowering is among the many other developmental processes that cytokinins have been reported to mediate in plants (Mok, 1994). Altered cytokinin concentrations before and after flower induction have been reported for some species (Lejeune *et al*., 1988). Intervention in the signal-transduction cascade causes by decreasing the cytokinin sensitivity in *Arabidopsis* resulted in a pleiotropic effect that includes the formation of a single, infertile flower (Deikman and Ulrich, 1995). This effect was more complex than a dose response; it has been demonstrated in *Arabidopsis* that the effect of an aromatic cytokinin on the flowering program is dependent on the developmental stage of the apical shoot meristem (Besnard-Wibaut, 1981; Venglat and Sawhney, 1966).

Dewitte *et al*., (1999) reported the considered cytokinin distribution in tobacco (*Nicotiana tabacum* L.) shoot apices in distinct phases of development using immunocytochemistry and quantitative tandem mass spectrometry. In contrast to vegetative apices and flower buds, it detected no free cytokinin bases (zeatin, dihydrozeatin, or isopentenyladenine) in prefloral transition apices. They also

observed a 3-fold decrease in the content of cytokinin ribosides (zeatin riboside, dihydrozeatin riboside, and isopentenyladenosine) during this transition phase. It concluded that organ formation (e.g. leaves and flowers) was characterized by enhanced cytokinin content, in contrast to the very low endogenous cytokinin levels found in prefloral transition apices, which showed no organogenesis. The immunocytochemical analyses revealed a differing intracellular localization of the cytokinin bases. Dihydrozeatin and isopentenyladenine were mainly cytoplasmic and perinuclear, whereas zeatin showed a clear-cut nuclear labeling. To our knowledge, this is the first time that this phenomenon has been reported. Cytokinins do not seem to act as positive effectors in the prefloral transition phase in tobacco shoot apices. Furthermore, the differences in distribution at the cellular level may be indicative of a specific physiological role of zeatin in nuclear processes.

2.3.3.3.2 Auxins

Auxins affect many developmental processes, including pattern formation in embryo development, induction of cell division, stem and coleoptile elongation, apical dominance, induction of rooting, vascular tissue differentiation, fruit development and tropic movements (Srivastava, 2002; Taiz and Zeiger, 1998).

In higher plants, indole acetic acid is synthesized by multiple pathways. In some, tryptophan serves as the precursor, whereas in others, the immediate precursors of tryptophan and indole-3-glycerol phosphate, seems to give rise to IAA by parallel pathways. Regulation of growth in plants may depend in part on the amount of free auxin present in plant cells, tissues and organs. Levels of free auxin can be modulated by several factors, including the synthesis and breakdown of conjugated IAA, IAA

metabolism, compartmentation, and polar auxin transport (Taiz and Zeiger, 1998). Indirect evidence for production of auxin-like substances in flowers is that near normal stem elongation can be restored following flower removal by auxin application to the cut surface. Auxin has long been implicated in many aspects of plant growth and development including flower development. However, the exact roles of auxin in flower development have not been well defined until the recent identification of auxin biosynthesis mutants. Auxin is necessary for the initiation of floral primordia, and the disruption of auxin biosynthesis, polar auxin transport or auxin signaling lead to the failure of flower formation. Auxin also plays an essential role in specifying the number and identity of floral organs. Further analysis of the relationship between the auxin pathways and the known flower development genes will provide critical information regarding mechanisms of organogenesis and pattern formation in plants (Youfa and Yunde hao, 2007).

Careful excision experiments demonstrated that ovules are the source of auxin moving into the stalk of *Fritillaria*. The first auxin peak coincides with intense cell division in the ovules and formation of the embryo sac (Bernier *et al*., 1981b). In many other species, the gynoecium is the major site of auxin production during flower development (Bernier *et al*., 1981b). Auxin production by anthers has also been reported, especially during microsporogenesis. The anthers would be the principal auxin source during the early stages of flower bud development, whereas the ovary begins to produce larger amounts of auxin at later developmental stages (Bernier *et al*., 1981b).

2.3.3.3.3 Gibberellins (GAs)

Relatively high GA levels have been found in flowers and inflorescences of several species as a function of stage of development. In *Mirabilis*, the amount of GA in the floral tube increases rapidly after the calyx becomes visible above the bracts 8 days before anthesis, reaches a maximum 3 days later, and then declines markedly. The stamens, especially the anthers, contain the largest quantity of GA and mainly account for the changes in concentration which occur during flower development. High GA levels are also found in petals and stamens of some other species. As, when male sex expression is inhibited, GA levels are frequently reduced not only in the flowers but also in vegetative tissues (Bernier *et al*., 1981b).

2.3.3.3.4 Ethylene

Ethylene production by the two upper shoots of *Baccara* rose plants, grown under full light during summer in Israel, is relatively high. With 50% shade, ethylene production decreases sharply in both shoots and remains low in the upper shoots (which all develop a terminal flower to anthesis). In contrast, ethylene production by the second shoots, which all become blind, increases by the second day following shading and by 4 days, where it is several times greater than that in the upper shoot (Bernier *et al*., 1981). We know now that flower atrophy in shaded rose shoots is probably controlled by some interaction between ethylene and reduced photosynthate transport rather than by ethylene level. Ethylene released by the abortive second shoots is about the same as that of the non abortive shoots exposed to full light so that the role of assimilates may include in that of determining bud response to ethylene (Bernier *et al*., 1981b).

2.3.3.4 Assimilates

Growth and development of different plant parts are affected by total assimilate production and partitioning among sink organs. Sink strength of various tissues is constantly changing during growth and development of the plant. During vegetative development, young leaves as well as vegetative plants are very strong sinks. When flowers or fruits are produced, assimilates are translocated to serve the developing flowers or fruits (Turgeon, 1989; Weaver and Johnson, 1985). Therefore, photoassimilate accumulation or diversion to plant organs, i.e., shoot meristems, might be related to size and complexity and to maturation. Environmental treatments that enhance growth rate and early flowering of juvenile plants are the same as those that enhance photosynthesis. It is well established that auxins, cytokinins, and GA promote the mobilization of assimilates by creating metabolic sinks, and may create competition among sinks for assimilates (Goldschmidt and Huber, 1992). Hackett and Sachs (1976) suggested that hormonal control of assimilate partitioning might be involved in phase changes. Franck (1976) showed that the change in morphological characters during phase change in several plants, e.g. leaf shape, and leaf or branch arrangement, was associated with an increase in the size of the shoot apical meristem. This was supported by the anatomical studies of Stein and Fosket (1969), that showed a large apical area in reproductive compared to vegetative English ivy. The implication is that during transition to the reproductive phase, the mature apex has greater competitive ability to attract assimilates than the vegetative apex. Therefore, Allsopp (1954) hypothesized that nutrient diversion caused alteration in the pattern of apical activity in the vegetative-to- reproductive transition.

2.3.3.5 Photoperiod

Flowering is a cascade reaction consisting of several steps. In the photoperiodic flowering, the photoperiodic signal is first received by phytochrome in the leaf, and the signal from phytochrome starts the biological clock. After the biological clock measures a certain period of time (inductive photoperiod), the production of the flowering stimulus in the leaf begins. The flowering stimulus is transmitted from the leaf to shoot apex. Upon arrival of the flowering stimulus, the growth mode of the meristem is changed from vegetative to reproductive. The shoot apical meristem produces primordia of floral organs; sepals, petals, stamens and carpels; thus generating a flower bud. All of these steps from the photoperception by phytochrome to the generation of a flower bud tend to be included in the concept of flowering. However, the perception of the photoperiodic signal is common to all processes of photomorphogenesis, such as seed germination, timing and direction of cell division, and stem elongation. The process of time measurement by a biological clock is also common to photoperiodic responses as seen in the dormancy of seed and bud, leaf abscission, bulb formation and others. These two processes are not steps specific to flowering itself. Only the production of the flowering stimulus and the response of the stem apical meristem to it are steps unique to flowering. The process between these two steps is flowering in a narrow sense (Kiyotoshi, 2003).

Photoperiodic flowering is not only a photoperiodic phenomenon but also a photomorphogenetic one. The studies on photoperiodism and photomorphogenesis now use more simple experimental systems such as ferns, single-celled algae and cyanobacteria. Therefore, flowering physiologists have concentrated their studies on flowering in the narrow sense, that is, floral induction and floral evocation. The main

theme in the studies of flowering has become the study on the flowering stimulus (Kiyotoshi, 2003).

Myoga (*Zingiber mioga* Roscoe) was grown under long-day a condition (16 hrs) and a short-day condition (8 hrs) with a night break produced flower buds, while those under short-day condition (8 hrs) did not flower (Stirling *et al*., 2002). *Suaeda salsa* formed flower buds and flowered in photoperiods of 8-14 hrs but not in 15 hrs or longer (Zhao *et al*., 2002). In *Tanacetun cinerariaefolium* L., day length had a quantitative effect on both flower initiation and development, where both processes were promoted by long-day (Brown, 1992). *Curcuma alismatifolia* is an economically important ornamental crop in Thailand. Plant flowers in July to August (12-13 hrs of day duration) and enters dormancy during winter (Nov to Dec) when the day 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. Investigation of the control of floral initiation and development by photoperiod is expected to be useful information for cut flower production, especially for off-season flowering.

2.3.3.6 Temperature

Plant processes are closely related to the environment, both during the dormant period and during the growth phase, allow accurate control of flowering and the production of the commercial end-product (Rees, 1992). Flowering process is controlled by environmental conditions and developmental regulation, and temperature is the major factor that affects growth and flowering of flower bulb

(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 (Kamenetsky *et al*., 2000). Temperature affects the rates of all plant processes. Within limit increased temperature speeds growth and development, but there are also specific effects of temperature on factors, such as flower initiation, dormancy breaking, and plant morphogenesis (Rees, 1992).

In addition, temperature also affects sensitivity of biochemical reactions of photosynthesis (Taiz and Zeiger, 1998). As the temperature of a plant decreases, there is a temperature at which a plant stops growing and developing. This temperature is called the base temperature, and varies widely among plants. As the temperature increases above the base temperature, plants grow faster, until it reaches its maximum rate of development. This is the optimum temperature and also varies among plant species. Plants that originate from warmer climates tend to have higher optimum temperatures than those from cooler climates. At temperatures above the optimum, the rate of plant development also decreases. This difference in optimum temperature among plant species makes it difficult to grow a variety of plant material with different temperature requirements in the same greenhouse (http://www.umass.edu/umext/floriculture/fact_sheets/greenhouse_management/gh_cool_growing.htm).

Phalaenopsis is usually grown at 28 °C to inhibit flower initiation, while a cooler night than day temperature regime (e.g. 25/20 °C day/night) is used to induce flowering (Blanchard and Runkle, 2006).

Flowering time of *Hippeastrum* can be controlled by applying specific thermal regime to large sized bulbs. Due to high-energy costs, the aim of this study

was to examine the possibility to reduce soil heating and keep high bulb growth rate by increasing the CO_2 concentration. Two sets of experiments were carried out in a controlled greenhouse at the North-Western Israeli Negev Desert. In both experiments, bulbs of different initial sizes were grown under two levels of CO_2 concentrations (ambient, 350 ppm and elevated, 1000 ppm) combined with different minimum soil temperature regimes. In the first experiment three temperature regimes (16, 22 and 24 °C) were tested, while in the second experiment only one minimum soil temperature regime (22 °C) was investigated. In both experiments, raising CO_2 concentrations from the ambient level to elevated one, or increasing soil temperatures resulted in a higher bulb growth rate. Temperatures, CO_2 concentrations and initial bulb size significantly influenced the final diameter of the bulbs. A significant difference in final bulb diameter was obtained only between the 16 °C treatment and the 22 and 24 °C treatments, but not between the two high temperatures tested. The area of the largest leaf was significantly affected only by the soil temperature treatments. No effect of CO_2 concentrations on leaf area development was detected. The number of leaves, however, was affected by the CO_2, but not by the temperatures. Bulbs grown under elevated CO_2 had a higher flowering rate than under ambient CO_2. This was effective both in shortening the period of time from re-planting until flowering and by the significant high number of flowers compared to the ambient CO_2 condition (Ephrath *et al*., 2001).

Day and night temperature

Efforts have concentrated on temperature control, which could result in well-shaped plants without a delay in flowering. The difference (DIF) between day

temperature (DT) and night temperature (NT) influences internode length, plant height, leaf orientation, shoot orientation, chlorophyll content, lateral branching and petiole and flower stalk elongation in plants. Internode length increases as DIF increases. The response of stem elongation to DIF is greater when DIF increases from zero to positive than from negative to zero DIF. DIF has the greatest effect on plant height during the period of rapid growth in determinant crops. Furthermore, the responses to DIF are rapid, and most plants respond to a change in DT and NT within 24 hrs. The effects of DIF on stem elongation and leaf expansion are a result of increased cellular elongation rather than division. Average daily temperature (ADT) can influence internode elongation in some species. Leaf unfolding rate is affected by ADT but not by DIF. (Myster and Moe, 1995). *Erodium cicutarium* (L.) dry matter production is greatest with day temperatures of 18 to 34 °C combined with night temperatures of 12 to 18 °C. A high night temperature at 24 °C is very detrimental, reducing dry matter production by 15 to 25% of that attained at 12 °C. Partitioning of biomass in leaves, stems and roots is markedly affected by day and night temperatures. Stem weight ratio is greatest at day temperatures of 18 to 34 °C and night temperatures of 18 to 24 °C. Maximum leaf weight ratio occurrs at day and night temperatures of 10 to 18 °C. Root biomass was little affected by day temperatures, but is greatest at a night temperature of 12 °C, declining substantially as night temperature increases from 12 to 24 °C (Blackshaw and Entz, 1995).

Experiments by McElroy *et al*., (2004), were conducted in environmental chambers to evaluate the effects of photoperiod and temperature on Florida betony (*Stachys floridana)* growth and development. Plants were exposed to two photoperiods, short day (9 hrs) and long day (9 + 3 hrs night interruption), and three

day/night temperature regimes, 18/14, 22/18, and 26/22 °C. After 10 wk of growth, shoot length and weight were 3.4 and 3.5 times greater in the long-day photoperiod and with the 26 and 22 than with the 22 and 18 °C day and night temperature regime, respectively. Shoot number, however, was greatest in the short-day photoperiod and at a lower temperature of 22/18 °C. Shoot number in long day with 22/18 °C and 26/22 °C environments increased asymptotically. No difference in root weight was observed between long- and short-day environments, but root weight increased with increasing temperatures. Flowering and tuber production occurred only in long-day environments, with greater production of both at higher temperatures. Results provided a general framework for understanding Florida betony growth and development characteristics in the field and provided insights what should be considered in developing control strategies (McElroy *et al.*, 2004).

One-year old scale bulblets of *Lilium longiflorum* Thunb. 'Nellie White' (Easter lily) were grown for 107 days during growth period 1 (GP-1) in growth chambers under six constant day/night temperature regimes of 30/26, 26/22, 22/18, 18/14, 14/10 and 10/6 °C. Subsequently, half of the plants in each temperature regime were transferred to 18/14 °C and the other half continued at the six constant temperature regimes. Both groups of plants were grown for an additional 89 days in growth period 2 (GP-2). Continuous temperatures of 26/22, 26/22–22/18 and 26/22–18/14 °C produced the greatest increase in basal bulb fresh weight (the main planted bulb), basal bulb circumference and stem bulb fresh weight, respectively. However, shifting these optimal temperatures to 18/14 °C during GP-2 resulted in a lower increase in basal bulb fresh weight and circumference. The optimum range for stem bulb production was expanded to 30/26–14/10 °C by shifting to 18/14 °C. The

greatest increase for basal root growth occurred at 14/10–10/6 °C and for stem growth was at 14/10 °C. The temperature shift did not affect either root type. The maximum increase for stem length was at 26/22 and 22/18 °C and for stem plus leaf weight was at 14/10 °C under constant temperature regimes. Transferring the plants from 10/6 to 18/14 °C resulted in the greatest increase in stem length and from 10/6 and 14/10 to 18/14 °C gave the greatest increase in stem plus leaf weight. The greatest increase in the number of leaves occurred at 26/22 and 10/6 °C, but this growth parameter was unaffected by shifting to 18/14 °C, indicating that leaf number was determined in GP-1. Bulbils developed only when bulbs from high GP-1 temperature regimes (30/26 and 26/22 °C) were transferred to 18/14 °C during GP-2. Lower temperatures tended to favor an increase in flower bud production under continuous temperature regimes, while shifting to 18/14 °C increased flower bud production after initially high and low temperatures. Meristem abortion was greatest at 30/26 °C followed by 26/22 °C, but was not affected by temperature shifts in GP- 2 Thus, it was concluded that the abortion was induced or initiated during GP-1 (Kim *et al*., 2006).

Flowering-sized bulbs of *Lachenalia* that developed under three different temperature regimes were used to assess the quality of subsequent pot plants. Plants were grown in a growth cabinet at a 15/10 °C day/night temperature regime. When the oldest flower of the inflorescences opened, the pot plants were transferred to a growth cabinet that provided a constant temperature at 22 °C with lower lighting conditions to simulate conditions. The flowering date, keeping ability, as well as, the morphology of the inflorescences were evaluated. After senescence of the inflorescences, the plants were harvested and dissected into different plant parts for

evaluation. The temperature pre-treatments had a major effect on the performance of the subsequent pot plants. Flowering occurred 8 weeks earlier as compared to plants normally grown in outdoor conditions in the Pretoria region (summer rainfall area). Furthermore, the low temperature regime treated bulbs produced inflorescences with the longest keeping ability and simultaneous flowering was noticed. The lower the temperature regimes during the bulb production phase, the greater were the peduncle length, rachis length, floret number, as well as, the peduncle diameter of the primary, secondary and tertiary inflorescences (Toit *et al*., 2004).