Chapter 2

Review of Literature

2.1 Origin and distribution of litchi

Litchi originated in southern China, northern Viet Nam, and Myanmar. Numerous wild litchi trees were found in moist forests in Hainan Island from low elevation up to 600 and 1,000 meter above mean sea level (masl) and below 500 masl in hilly areas of the Leizhou Peninsular, west Guangdong and east Guangxi in China as well as in parts of Vietnam, north of Hanoi (Menzel, 2002b).

China has a long history of litchi cultivation for more than 2,000 years. Litchi reached Myanmar by the end of 17th century and it was introduced to India and Thailand about 100 years later. It reached Madagascar and Mauritius around 1870 and it was introduced to Hawaii in 1873 by a Chinese trader. It was distributed from India to Florida between 1870 and 1880 and it was introduced in California in 1897. It was probably brought to Australia in 1954 and reached Israel sometimes during 1930-1940 (Mitra, 2002). However, introduction of litchi to Thailand must be earlier than 150 years ago because it was very likely that litchi fruits came firstly along with Chinese traders and immigrants during the Ratanakosin Era (since 1782) (Sethpakdee, 2002). The fruits are very popular in China and Southeast Asia, however, it is less well known in Africa, the Middle East and America (Menzel, 2002b).

2. 2 Climate of litchi growing area

Litchi commercial growing areas in Asia and Australia are usually found in subtropics from 17 to 30 degrees latitude. A few small industries are also based at 300-600 masl in tropical locations in central part of Thailand and in a few selected areas of the Philippines and Indonesia. Most of the sub-tropical areas have cool winters and warm summers. Most of litchi commercial areas have winter below 20°C. Winters are dry, with rainfall of less than 50 mm. The temperature during fruit set is usually between 20° to 30°C. The rainfall is usually less than 50 mm in spring. Summers are warm to hot (28-33°C). Average summer rainfall is at least 150 mm. Near equatorial areas (latitude 11°N; elevation 9 m), temperature do not fall below 20°C

during the year and yields are very unreliable, even though there is a distinct dry season (Menzel, 2002b).

2.3 Litchi in Thailand

In Thailand, the litchi cultivars are divided into two groups; lowland or tropical cultivars and low temperature or sub-tropical cultivars. The lowland cultivars were commercially grown in the central region (14°N), require moderately low temperature (Yapwattanaphun and Subhadrabandhu, 2001; Sethpakdee, 2002) and a drought period for flowering (Anupun and Sukhvibul, 2003). More than ten cultivars are known in this group and 'Kom', compact canopy size, is the most famous cultivar among the others, e.g. 'Kralok Bai-Yaow', 'Sampao Kaew', 'Kiew Waan', 'Dang Payom', 'Kratone Tong Pra-rong', etc. Recently, 'Pantip', a new litchi cultivar has been found and grown mainly in Kanchanaburi province.

The subtropical cultivars, which require a longer cold period for flowering, are commercially grown mainly in northern part of Thailand. Chiang Mai (8,322 ha) and Chaing Rai (5,763 ha) are the two major provinces that contribute more than 60 percent of the overall acreage (22,937 ha). There almost ten cultivars are known such as 'Hong Huay', 'Chakrapad', 'Kim Cheng' ('Wai Chee' in Australia) and 'O-Hia'. 'Chakrapad', a cultivar with the largest fruit size, usually receives the highest price (Yapwattanaphun and Subhadrabandhu, 2001; Sethpakdee, 2002).

The production areas range from the tropical lowlands of the central region including eastern and western regions to the sub-tropical climate of the north and northeast regions. The fruiting season could lengthen up to a full three months period. Earliest harvesting can be from mid-March in the central region, last fruiting in the northern region takes place in mid-June (Table 2.1). There are several areas where selected cultivars could fill up any gap of supply throughout this three months period. Thus better confidence in supply could strengthen marketing planning strategies (Sethpakdee, 2002).

Table 2.1 The harvesting time of litchi in Thailand separated by the production zone

Production zone	Month			
	March	April	May	June
Chanthaburi	———			
Samut Songkhram	———			
Kanchanaburi	———			
Nakhon Ratchasima	———			
Phayao		———		
Chiang Mai and Chiang Rai			———	

2.4 Botany of 'Hong Huay' litchi

'Hong Huay' cultivar, the most widely grown cultivar, is grown in northern part of Thailand (Figure 2.1 A), in China and in Australia. It is also known in other names such as 'Kwai Mi' and 'Charley Tong' in Hawaii, 'Kwai Mi' and 'Tai so' in Queensland, 'Tai So' in China (Figure 2.1 B), and 'Mauritius' in South Africa (Aradhya *et al.*, 1995).

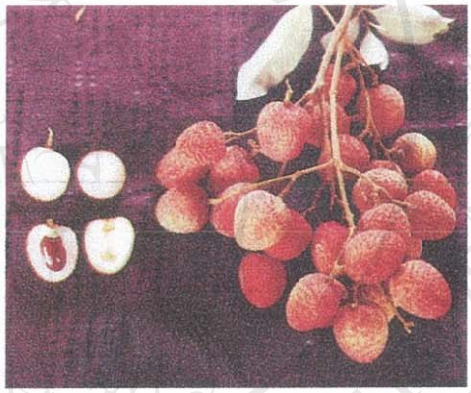
Compound leaves of 'Hong Huay' are medium to large, about 26 cm wide and 25 cm long, with 3-4, usually 4 pairs of leaflets. Petioles are dark green in adaxial and green in abaxial, with a thickness of 0.24 cm (Figure 2.2 B). Leaflets are dark green, oval-shaped and small to large in size, i.e. 4 cm wide, 14 cm long and 0.09 cm thick, with undulate margin, cuspidate apex, acute base and smooth surface.

Flowering habit is profused with cream florets. Panicles are small to large, around 9 cm wide and 17 cm long (Figure 2.2 D). Fruit are ellipsoid in shape, medium in size, 3 cm in width, 3.2 cm in length and 17 g in weight. The red skin weights 3 g per fruit. The aril is dull white and about 10 g with 14.8% TSS. Seeds are ellipsoid in shape, 1.41 cm in width, 2.4 cm in length and 3.5 g in weight (Figure 2.2 E) (Yapwattanaphun and Subhadrabandhu, 2001).

A1 Canopy of 'Hong Huay' litchi

A2 Fruit clusters of 'Hong Huay' litchi

B1 Canopy of 'Tai So' litchi



B2 Fruit clusters of 'Tai So' litchi

Figure 2.1 Canopy and fruit clusters of 'Hong Huay' (A) and 'Tai So' (B) litchi (Plant Variety Protection Office, 2003)

Figure 2.2 'Hong Huay' litchi tree (A), young leaves (B), hermaphrodite functioning as male (C1) and female flowers (C2), panicles (D) and fruit clusters (E)

2.5 Development of litchi tree

2.5.1 Leaf development

Vegetative growth occurs as a series of flushes alternating with periods of rest. Flushes last for shorter periods and follow one another more rapidly at 25-30°C than at 15-20°C. Regular watering and fertilizing promote leaf growth under the warmer temperatures (Menzel, 2001). Shoot elongation is extended by repeated flushes, during which several leaves and internodes expand. At the end of leaf expansion, the leaves thicken and change from light to dark green. The minimum interval between successive vegetative flushes (or between vegetative and floral shoots) is approximately six weeks, but the dormant buds generally require at least four weeks to flowering. However, the interval can be longer, depending on the weather and the physiological state of the plant. An interval between successive flushes is increased by low temperatures, low light, drought, and nutrient deficiencies (Menzel, 2002b).

2.5.2 Flower development

The inflorescence as a determinate is composed of several multiple-branched panicles initiated on the present season's wood. The panicles are normally produced terminally in clusters of ten or more, although in some trees, a high proportion of axillaries may be produced. Inflorescences are generally mixed, with the lowest buds producing leaves only, the middle buds producing floral buds in the axils of the leaves. Moreover, the topmost buds producing only floral branches and sometimes very small leaves which do not persist. The pattern of development is related to differences in temperature experienced during early shoot development. Growth of the inflorescences is usually complete in six to twelve weeks (Menzel, 2002a).

2.5.3 Anthesis

Flowers normally open for 20 to 45 days within an individual orchard and cultivar, depending on cultivar and seasonal conditions. Flowering is more compact when it occurs late in spring in warm weather. There is no pollination unless the male and female stages overlap. Generally, the last stage of male flowering provides most of the pollen for the female flowers. Flower opening occurs during both day and night, with peak opening in early morning, provided temperatures above 15°C. Flowering is usually the best with at least three weeks of low

temperatures. Male flowers shed pollen for three days after opening, which do not occur at the same time. Pollen is lasting no more than a day after shedding and individual female flowers have also a limited life. Extended hot or dry weather can dry out the stigmas. However, one to ten percent of the female flowers carry fruits to harvest (Menzel, 2002a).

However, in 'Hong Huay' litchi, the panicles have three types of flowers as following: male flower (Type I), hermaphrodite functioning as female flower (Type II), and hermaphrodite functioning as male flower (Type III) (Figure 2.2 C). A period of flower blooming, from the first opening flower until the last flower, is about 24 days, which it also depends on the size of inflorescence. Generally, there are two generations of blossoms. Firstly, type I flowers begin to bloom then type II and type III flowers are blooming respectively. After the first flowering, the litchi trees may stop to flower again in the next generation. However, at the second generation of litchi flowering, there are very less of flowers than the first. It is started with type I, II and III, respectively as same as in the first generation (Sethpakdee, 1997).

2.5.4 Fruit growth

Normally only one of the two ovaries of the female flower develops into a fruit. Depending on the season and cultivar, fruit take about 12 to 16 weeks to reach its mature stage. Fruit growth is normally faster during warmer weather (Menzel, 2002a). In Thailand, fruit growth pattern follows a simple sigmoid curve with the total of 100-130 days from flower opening to fruit harvesting, which is also depended on the climate condition. The seed and fruit skin are firstly formed. The fruit skin develops continuously until harvesting stage, whereas seed growth will stop after three months of the fruit development. After that the aril is formed, which is coinciding with the fruit size (Sethpakdee, 1997). For exact information of developmental timing, Menzel (2002a) reported that not all parts of the fruit develop at the same time. During the first seven to eight weeks after fertilization, the fruit skin, embryo and seed skin are formed. After that, two to three weeks, the cotyledons included with most of the seed are formed, and the development of aril begins. At the end of this stage, the aril is about a third of fruit fresh weight. The final period of fruit growth is dominated by rapid growth of the aril. At fruit maturity, the aril is about 65 to 75 percent of fruit weight. In most cultivars, the skin color changes from green

to yellow-red to red with advancing maturity. This change is associated with a flattening of the skin segments and protuberances, an increase in sugar/acid ratio and eating quality.

2.6. Physiology of flowering

The transition to flowering is an important event in a life of a plant when the plant switches from vegetative to reproductive growth. Developmental signals that cause this transition originate outside a shoot apical meristem (SAM), and the SAM remains uncommitted to flowering prior to its perception of external signals (Colasanti and Sundaresan, 2000).

2.6.1 Models of flowering

According to many research results, three theories concerning mechanism of flower initiation have been proposed. These theories attempted to explain the chemical control of the transition to flowering (Bernier, *et al.*, 1993; Levy and Dean, 1998; Macháckovά and Krekule, 2002).

2.6.1.1 Florigen/antiflorigen model

The transition from vegetative to floral development in angiosperms was proposed to involve interactions between florigen as a floral-promotive signal and antiflorigen, a floral-inhibitory signal (Kulkarni, 1991; Bernier *et al.*, 1993).

This model in mango proposes by Kulkarni (1991) that an interactive role for the putative floral stimulus in leaves and an inhibitor residing in leaves and fruits. During the period of dormancy following a vegetative cycle, genetic and environmental factors determine the level of synthesis of the putative floral stimulus. Flowering follows only if certain correlative factors are present. For example, flowering will only happen if the receptor bud becomes active. If fruits are recently present on the stem, vegetative growth will occur. Presence of the putative inhibitor in leaves interferes with expression of the floral stimulus resulting in vegetative growth. Finally, the level of the floral stimulus determines the response; high levels give rise to normal panicles, intermediate levels give rise to mixed panicles and low levels result in vegetative growth.

Such as in 'Kensington' mango, floral initiation occurred after a 2-3-month rest period during autumn/winter when a critical threshold level of carbohydrate was reached in buds

together with a putative floral stimulus. Bud break under cool winter/spring temperature showed a high percentage of terminals flowering with fruit set suppressing vegetative flushing on individual fruiting stems until after they had matured and been harvested (Searle *et al.*, 1995).

Branches bearing flowering shoot of 'Keitt' and 'Tommy Atkins' mango were girdled and/or defoliated by using different lag periods to disrupt transport of the floral stimulus and prevented its production. Only girdled branches without defoliation produced inflorescences. It was indicated that inflorescence differentiation involved a putative floral stimulus, which generated in mature leaves and translocated in the phloem (Nunez-Elisea *et al.*, 1996). Similar to longan experiment, the finding result showed that floral stimulus originated in mature leaves and translocated *via* the phloem to apical bud (Sruamsiri *et al.*, 2003).

2.6.1.2 Nutrient diversion model

This model was proposed that inductive treatments resulted in an increment the amount of assimilates moving to the apical meristem, which in turn inducing flowering (Sachs, 1977; Chacko, 1991; Davenport and Nunez-Elisea, 1997; Levy and Dean, 1998; Corbesier *et al.*, 1998). The fundamental principle is assumed that yield is the product of photoassimilate (carbohydrate) accumulation and subsequent redistribution during the annual growth cycle. Accumulated photoassimilates are proposed to drive critical growth events which require higher levels of resources than are available from current photoassimilation (Davenport and Nunez-Elisea, 1997).

Moreover, Chacko (1991) has proposed a flowering model driven by assimilate supply and diversion to apical meristems. Environmental conditions, such as water stress, cool temperatures, high evaporative demand, flooding, girdling and other intrinsic or extrinsic events which cause a check in vegetative growth, result in a shift in carbohydrate partitioning and a diversion of soluble assimilates to shoot apices. This elevated carbohydrate status in buds, together with a floral stimulus results in floral induction. Vigorously growing cultivars and juvenile plants have low starch reserves (Whiley *et al.*, 1991) and a diversion of soluble assimilates from shoot apices results in floral inhibition (Davenport and Nunez-Elisea, 1997).

Sinapis alba plants exposed to a single short day plant at an irradiance 2.5 times higher than normal did not flower. However, this treatment caused an increment of sugar levels and acid invertase activity in the apical meristem as well as some ultrastructural changes that are typically

observed during the transition to flowering (Havelange *et al.*, 2000). This effects which were due presumably to increase of photosynthesis and assimilate availability, emphasize the possible role of carbohydrates in the control of this transition (Davenport and Nunez-Elisea, 1997).

2.6.1.3 Multifactorial control model

A number of promoters and inhibitors, including phytohormones and assimilates, were involved in controlling the developmental transition. This model attempted to account for the diversity of flowering responses. It was proposed that different factors could be limiting for flowering in different genetic backgrounds and/or under particular environmental conditions (Bernier, 1988; Nunez-Elisea, *et al.*, 1990, Levy and Dean, 1998; Gibson, 2000).

This model is based on many experimental evidences (Davenport and Nunez-Elisea, 1990; Nunez-Elisea *et al.*, 1990) as well as on research with other tropical and subtropical crops with similar phenological cycles (Menzel, 1983; Menzel and Simpson, 1994). Focusing on events occurring in individual buds, it is including monoembryonic and polyembryonic cultivars growing in both tropical and subtropical climates and attempts to explain the physiological basis for the annual progressing of the phenological cycle. Moreover, Machackova *et al.* (1996) stressed that changes in the level and transport of endogenous hormones and saccharides were found during flower induction and they were possible involved in the regulation of flowering. In addition, Bernier *et al.* (1993) indicated that photoperiod and irradiance were perceived mainly by mature leaves in intact plants. Temperature was perceived by all plant parts, although low temperature (vernalization) was perceived by the root system. These signals were generally transported from leaves to the apical meristem in the phloem with assimilates. And the signals originating in roots were presumably transmitted in the xylem with the transpiration stream.

2.6.2 Flowering process

During vegetative development, the shoot apical meristem generates leaf primordial directly from its flanks in a stereotypical spatial arrangement or on the periphery of a vegetative meristem. Floral meristems are small, spherically shaped mounds of cells, which produce four types of lateral organs in concentric ring call whorls. Sepals are initiated first in the outermost whorl, followed by petals in the second whorl, and stamens in the third whorl. The floral

meristem is then consumed in the formation of the central carpels, which form the gynoecium that ultimately encloses the seeds of the next generation. Thus, the floral meristem is eventually terminated, whereas the SAM grows indefinitely (Fletcher, 2002).

However, a transition to flowering involved major changes in a pattern of morphogenesis and cell differentiation at SAM flowering involves several processes that occur in different parts of the plant. Once the meristem becomes committed to the new developmental program (flowering in this case) (Taiz and Zeiger, 1998). However, many researchers defined steps of flowering process in different ways such as reviews below.

Wellensiek (1977) suggested that the principles of flower formation could be defined as anything which happened between flower-forming genes and meiosis. The beginning of the process referred to an induction. It was followed by differentiation of growing point (initiation) and later, by differentiation of flower primordial. Each of these processes was distinct and required specific internal and external conditions to proceed.

During early plant growth, leaf primordial is produced on the periphery of a vegetative meristem at the shoot apex. When plants are induced to flower, two new types of meristem are usually produced. First, the apical meristem switches to become an inflorescence meristem. Second, floral meristems are produced, often arising as small bulges on the periphery of the inflorescence meristem (Coen and Carpenter, 1993).

Furthermore, Hopkins (1999) defined that flowering was conveniently divided into three stages. Floral induction referred to events that signal the plant to alter its developmental program. SAM reorganized to produce floral primordial rather than leaf primordial. Floral evocation referred to events that commit the meristem to formation of flower primordial in place of leaves. When floral developmental program continued, floral primordial would arise on the flanks of the apical meristem in place of leaves. The primordial continue to enlarge, giving rise to primordial for separate floral structures and finally a mature flower.

Colasanti and Sundaresan (2000) indicated that floral induction caused a cascade of processes within the SAM that resulted in its restructuring, accompanied by changes in the rate and pattern of cell division, and the formation of floral structures instead of leaves. Moreover, floral evocation refers to events a mitotic activity also increases in the apical meristem before a differentiation of the flower bud (Thomas, 1963).

The transition from vegetative to flowering shoot apex is accompanied by dramatic changes not only in the structure of the apex, but also in patterns of cell division and growth and the nature of derivatives. The tunica-corpus organization of the vegetative shoot meristem is lost and cell divisions spread throughout the apical meristem. Instead of leaves and buds being formed, floral parts-sepals, petals, stamens, and pistils are initiated and there is little elongation growth between initiations of these parts. The floral parts are borne on a condensed axis, and the flower is a condensed shoot. The shoot (or floral) apex is used up in the formation of the floral parts and its meristematic potential is terminated. Floral parts like leaves are determinate organs, and the flower is a determinate shoot (Srivastava, 2002).

In fruit trees, flowering process especially the stage of floral differentiation was shown various morphological characters such as in mango. Nunez-Elisea *et al.* (1996) stressed that development of the basal nodes was determined separately from that of the apical meristem as the occurrence of unique transitional phenotypes. Furthermore, Tongumpai and Ogata (2000) defined a differentiate stage that floral primordial was the first visible at the axil of the primordial and later developed toward the apex. At later stages a number of floral buds were found at the axil of leaf primordial.

Furthermore, in 'Hass' avocado, Salazar-Garcia *et al.* (1998) revealed that a primary axis meristem changed a shape from convex to flat to convex during transition of flowering. Then the bracts and their associated secondary axis inflorescence meristems were at stage of floral initiation.

In 'Shixia' longan, Qiu *et al.* (2001) reported that an axillary meristem develops into a lateral inflorescence before the apical bud differentiated. Flower bud differentiation did not occur until shoots elongated. The morphogenesis of apical and axillary meristems proceeded independently with leaves in some inflorescence. An axillary inflorescence developed when leaflets were removed and the terminal bud stopped growing.

In litchi, Shukla and Bajpai (1974) indicated that a primordial on the elongated main axis appeared as the first indication of floral differentiation. The emerging inflorescence was similar in appearance to a vegetative flush. It may be possible to identify a flush as a developing inflorescence when primordial of the secondary inflorescence branches occurred in the axils of the small leaves. However, Chen (1991) suggested that the criteria of flower bud initiation were

used as the flattening and elongation of the apical meristem and a concurrent appearance of flower primordial.

Furthermore, Huang and Chen (2003) reported that floral induction occurred when the apex meristem was quiescent in litchi, whereas floral initiation started from the "millet stage" when cell division in the apex meristem became active. "Whitish millet" as symbolized the 'demarcation' separating floral induction and floral initiation. The slow growing buds in the leaf axils appearing as "whitish millets" had acquired the potential for further differentiating and developing into flowering panicles under ascending air temperatures and moderate moisture.

In addition, Sruamsiri (2004, personal communication) theoretically divides flowering process in litchi into five stages, i.e. induction, initiation, differentiation, inflorescence protrusion and anthesis. In induction stage, gene expression, RNA, cytoplasm and enzymes change occurs in bud. In following stage, cells in terminal apical meristem become active and may be enlarge accompanying with change in endogenous hormones balance in the initiation stage. Differentiation starts when cells response to those chemical changes in cytoplasm and forms leaf or inflorescence primordial, floral organ can be detected under stereomicroscopy and terminal bud swelling. Inflorescence bud then emerges and elongates to become the visible panicles in the protrusion stage. Sexual development still can be occurred or reversed when temperature change or with chemical treatment. Then flowers bloom as anthesis stage.

However, in this experiment, the flowering process in litchi is divided into only three stages, i.e. floral induction, differentiation and anthesis. The floral induction stage refers to events, which start at a quiescent apex meristem during inducing condition following an active-swell apical bud occurs under stereomicroscopy at a final stage. Whereas the floral differentiation refers to events that first inflorescence meristems/buds in the apex become visibly under stereomicroscopy and these meristems develop into floral buds following a panicle also progresses at a final stage. Then a number of floral buds bloom at stages of anthesis.

2.7 Factors affecting flowering in fruit trees

In general, plant responses to endogenous physiological factors or environmental factors consist of three main events: perception of the stimulus, generation and transmission of signals and subsequent changes in downstream biochemical processes (Salinas, 2002). The interactions

of these endogenous and external factors enable plants to synchronize their reproductive development with the environment (Taiz and Zeiger, 1998).

2.7.1 Environmental factors

2.7.1.1 Effect of day length or photoperiod

Plant responses controlled by day length are numerous appearances. They are included the initiation of flowering, asexual reproduction, the formation of storage organs and the onset of dormancy. In the case of photoperiodism, transmissible signals from the leaves which referred to the floral stimulus, are translocated to the shoot apical meristem. However, day length alone is a doubtful signal. One is the coupling of a temperature requirement to a photoperiodic response. Certain plant species, such as winter wheat, did not respond to photoperiod until after vernalization had occurred (Taiz and Zeiger, 1998). However, Stern (1992) indicated that litchi was a day-neutral plant because an extremely short (8 h) or long (16 h) days did not affect its flowering at all. Similar to the studies of Stern and Gazit (2003), 'Mauritius' and 'Floridian' litchi were kept for 3.5 months (mid October to end of January) in the phytotron in Israel. Litchi plants were grown at two temperatures regimes, 22/12°C and 22/17°C day/night about 10 h at the high temperature and 14 h at the low temperature. Each temperature regime plants were conducted at short and long day length, 8 and 16 h, respectively. The finding showed that no effect of day length was found because the plants kept at 22/12°C flowered profusely under both short and long day conditions.

2.7.1.2 Effect of low temperature

Low temperature is an important factor affecting plant performance and distribution (Larcher, 1995), especially inducing flowering in responsive plants such as avocado, mango, olive, longan and litchi. In addition to the effect of low temperature on floral induction of subtropical fruits, many physiologists also observed the effect of root temperature on the shoot dormancy such as in mango, avocado and litchi. In avocado, temperatures below 25°C were essential for flowering and could not be replaced by water stress (Chaikiattiyos *et al.*, 1994). In mango trees, temperature at around 15°C for 30 days was required to promote inflorescence morphogenesis (Batten and McConchie, 1995; Nunez-Elisea and Davenport, 1995; Nunez *et al*,

1996). Nunez-Elisea and Davenport (1995) also reported that cool temperature rather than a short photoperiod caused floral induction, whereas warm temperature (near 30°C) rather than a long photoperiod prevented floral initiation of induced buds. However, Chaikiattiyos *et al.* (1994) reported that temperatures below 20°C were essential for the flowering of mango trees and could not be replaced by water stress.

Several researchers had been studying the effects of low temperature on litchi. The results repeated the evidences that litchi trees required low temperatures of around 15°C to flower successfully. Temperature may be varied among cultivars (Menzel and Simpson, 1994, 1995; Stern and Gazit, 2003; Huang and Chen, 2003). Temperature may effect directly on dormancy or on shoot development (O'Hare, 2002).

Moreover, Menzel and Simpson (1994) indicated that under the marginal temperature regime of 20/15°C day/night temperature, flowering occurred in seven litchi cultivars which its success fluctuated between 11 and 99 percent. Through similar result, Huang and Chen (2003) emphasized that chilling was more effective in a continuous pattern as compared to an alternating one. Hou-Bin and Hui-Bai (2003) repeatedly studying the floral induction phase of litchi over the period of 1998 to 2001, and reported that the floral induction effect was closely related to mean of maximum temperature and accumulated hours below 10°C between December 1 and January 15. Maximum temperature in "on" years was below 20°C whereas that in "off" year was above 21°C. Accumulated hours below 10°C in 'on' year was about 160 h whereas that in "off" year was 82 h. Moreover, O'Hare (2004) also suggested that both of root and shoot temperature had influence on litchi floral initiation. Low root temperature had a direct effect on the period of shoot dormancy which accompany with flower inducing, while high root temperature at 28/23°C inhibited it in litchi. However, an extended period of dormancy in itself was not sufficient for floral initiation, as low shoot temperature is a necessary prerequisite, too. Menzel *et al.* (1989) also reported that low root temperatures, 12.5°C, increased flowering in 'Tai So' litchi, whereas high root temperatures, 27.5°C, reduced flowering. In 'Chakrapad', Sritontip (1996) reported that litchi trees, grown under 15°C of root temperature regime, had better flowering than under normal root temperature (around 26°C).

About the effect of a cool period on floral inducing in litchi, Menzel and Simpson (1988) found in litchi trees grown under 15/10°C and 20/15°C day/night temperature regimes that the

panicles emerged after only four weeks and six weeks, respectively. Menzel and Simpson (1995) also reported that 'Wai Chee' cultivar needed ten weeks at the constant temperature of 15°C. In the studies with 'Calcuttia' and 'Rose-Scented' at Kanpur India, the first signs of floral differentiation occurred at about three to four weeks after the minimum fell below 10°C (Menzel, 2002b).

2.7.1.3 Effect of drought and water stress

Drought and water stress, natural environmental change, usually affects on the physiology of plants which the response differs among species of plants. Chaikiattiyos *et al.* (1994) studied on the effects of temperature and water supply on floral induction in tropical fruit trees. It was found that water deficits did not induce flowering in litchi, mango and avocado, whereas low temperatures did. On the other hand, the lemon flowered in response to water deficits but low temperature did not. The temperatures below 25°C for avocado and below 20°C for litchi and mango were essential for flowering and could not be replaced by water stress.

Promotion of autumnal water stress is one technique being used in the commercial litchi orchards in order to induce profuse flowering (Menzel and Simpson, 1994; Gazit, 2001; Stern and Gazit, 2003). In litchi, Menzel (2002b) indicated that drought could be used to control flushing patterns and improve flowering in localities with dry winters such as India and Thailand. However, no evidence had been found that dormancy induced by water stress in the absence of cool temperature was able to induce flowering of litchi (Menzel and Simpson, 1990; Chaikiattiyos *et al.*, 1994). On the other hand, flowering was favored by a dry period before and during floral initiation (Stern and Gazit, 2003).

The sequence of floral formation in litchi exhibited obvious phase changes and different requirements. Bud dormancy by moisture stress was the prerequisite for shoot maturation and the ensuring floral induction under chilling temperatures (Huang and Chen, 2003). However, water stress was neither essential nor sufficient for flower induction. Under inductive temperature regimes, full flowering occurred even in well-watered plants (Chaikiattiyos *et al.*, 1994). In addition, Sinlaphasomboon, (1994) suggested that leaf water potential needed not to be decreased to the lowest level for flower induction in 'Hong Huay' litchi. However, under reduction in leaf water potential together with decreasing temperature, high total non-structural carbohydrates

(TNC) and low total nitrogen content would be able to promote flowering in litchi. However, excessive water stress may result in a significant reduction in assimilation rate, chlorophyll destruction, root death, and leaf drop (Stern *et al.*, 1993; Menzel and Simpson, 1994; Menzel *et al.*, 1995; Roe *et al.* 1995).

Among the environment factors as described above, although low temperature (around 15°C) strongly effect on enhancing flowering in litchi, however, this steady low temperature under natural condition is very hard to achieve. That is the major problem causing irregular or even alternate bearing in litchi. Therefore, drought in winter prior to flowering is also helpful to induce flowering in litchi.

2.7.2 Endogenous factors

2.7.2.1 Juvenility

Litchi tree propagated from seed requires more than six years of age to produce flower, while those grown from air layering ready to bear fruit within 1-2 years. Flowering in the mature litchi trees usually occurs in mature terminal shoots, which experience a duration low temperature conditions (Manochai and Jarassamrit, 2000). In addition, Davenport and Nunez-Elisea (1997) showed the importance of shoot age on the ability to flower, especially in the low-latitude tropics, while the importance of low temperatures for flowering in trees grown at higher latitudes. Zheng *et al.*, (2001) reported that both 'Mauritius' and 'Brewster' litchi shoots should be about 15 weeks-old to response to cool night winter and initiate flowering. Moreover, Stern and Gazit (2003) confirmed that only flushing or no flowering occurred when young immature shoots were exposed to flower inductive temperatures.

Similar effects of low temperature and shoot age were also reported, e.g., in 'Tommy Atkins' mango, Nunez-Elisea and Davenport (1995) found that floral initiation occurred after at least 3 weeks of cool temperature treatment. The minimum leaf age at 7 weeks-old was necessary to showed response to cool temperature and floral inductive. In longan, Manochai *et al.* (2003) also found that girdling at the stage of mature leaf induced 76-78 % flowering at 23 days after girdling, while the girdled trees at the semi-mature leaf stage had only 65% flowering at 40 days after treatments.

2.7.2.2 Leaf photosynthesis aspects

Photosynthetic activity in the intact leaf is an integral process that depends on many biochemical reactions. Urban *et al.* (2004) indicated that the carbon status played a key-role in flowering and fruiting of mango trees, while influxes of carbohydrates seemed to influence fruit size and taste. Previous studies have shown the importance of the temperature response on leaf photosynthetic capacity, and the existence of inter-specific differences among responses obtained between different species or under differences environmental conditions (Dreyer *et al.*, 2001). In mango, Nir *et al.* (1997) suggested that mid-day quantum efficiency of PS II photochemistry values were highest after the warmest night. The values dropped down to 0.23 after the coldest night. Chilly night also caused the reduction of CO_2 uptake capacity and stomatal conductance.

Similarly, Allen *et al.* (2000) measured gas exchange of 'Tommy Atkin' scions on 'Turpentine' rootstock mango, which the leaves maintained under constant conditions for the CO_2 assimilation for 2 days. The results showed that large depressions in light-saturated CO_2 assimilation during a subjective night were primarily result of stomatal closure. The midday increased in stomatal limitation of CO_2 assimilation appeared to be the result of altered guard cell sensitivity to CO_2 following the dark chill. The mechanisms behind the dark chill-induced inhibition of photosynthesis need to account for a delay of a few hours after the chill, when control rates of photosynthesis were observed. This depression was associated with a rise in stomatal limitation of CO_2 assimilation and a decrease in Rubisco activity.

For the chemical affected on photosynthetic aspect, Sritontip *et al.* (2003) showed that longan treated with $KClO_3$, KNO_3 and NaOCl had more efficiency of PS II and net CO_2 assimilate rate than trees treated with thiourea and untreated in the 1st week before terminal bud break. The soil drench of $KClO_3$ and sodium hypochlorite had greater rate of net CO_2 assimilate in terminal bud break and the 1st week after terminal bud break.

The average daytime stomatal conductance (*Gs*) in 'Bengal' litchi increased from 100 to 220 mmol m^{-2} s^{-1} from August to December as soil temperatures rose about 13-26°C. The increasing of seasonal *Gs* was correlated with soil temperature, but less with leaf temperature. Higher *Gs* combined with higher potential evaporation in summer lead to lower midday leaf water potential than in winter (Andersen, 1989).

Nevertheless, Chaikiattiyos *et al.* (1994) stressed that net photosynthesis in lemon and litchi declined with increasing water stress reaching zero with a midday leaf water potential of -3.5 to -4.0 MPa. This decline in carbon assimilation appeared to be almost entirely due to stomatal closure.

2.7.2.3 Carbohydrates

A considerable proportion of the dry matter produced through photosynthesis is deposited in cell walls as cellulose, hemicelluloses and lignin. Therefore, it is not available for further utilization like starch and soluble carbohydrates. Apart from starch, soluble sugars namely sorbitol, sucrose, glucose and fructose are reported to be the main components of total non-structural carbohydrate (TNC) (Reich, 1985).

Jackson and Sweet (1972) believed that increasing carbohydrate at the site of flower formation could enhance flower bud formation in temperate trees. However, Lejeune *et al.* (1993) indicated that the early extra sucrose seemed to arise not from increased photosynthesis, whereas it from mobilization of reserve carbohydrates stored both in leaves and stem.

Several studies of carbohydrates concentration were investigated on the sub-tropical and tropical fruit trees such as longan (Pichakum *et al.*, 2003), litchi, lemon, mango and avocado (Chaikiattiyos *et al.*, 1994). Pichakum *et al.* (2003) investigated on longan cv. 'Daw' during 8 weeks before anthesis. The result showed that contents of TNC and reducing sugar (RS) in terminal shoots significantly increased during developing period of inflorescences.

Moreover, Chaikiattiyos *et al.* (1994) studied on temperature and water stress affecting starch concentration in root. The findings were indicated that the starch concentration in the roots of avocado, lemon, litchi and mango were generally higher at 18/15°C and 23/18°C than at 29/25°C. Water stress increased the root starch level in lemon and litchi, but decreased in avocado. There was no effect in mango. Moreover, there was a weak relation between a number of flowers per tree and the root starch concentration in lemon. In contrast, starch did not appear to control flowering under various temperatures and water regimes in the other species.

2.7.2.4 Plant hormones

1) Cytokinins (CKs)

Cytokinins are adenine derivatives with an isoprenoid side chain and play an essential role in plant development. Several CKs occur naturally in plants, however, zeatin, specifically trans-zeatin (Z), is the most abundant. The synthetic pathway of CKs in higher plants has been unclear and controversial for a long time, but progress finally seems to be achieved with the cloning of genes encoding isopentenyl transferases (IPTs) in *Arabidopsis*. These IPTs seem to use ATP/ADP, rather than adenosine-5'-monophosphate, together with dimethylallyl diphosphate, to yield isopentenyladenine monophosphate, which ultimately gives rise to zeatin and other naturally occurring adenine cytokinins. The possibility that CKs may arise from the degradation of tRNAs is not considered likely. Ribosylated derivatives, with a ribose or ribose 5'-monophosphate attached to the adenine moiety, of CKs are common. Conjugates of CKs where a glycosyl moiety is attached to the OH group in the side chain are also common. Both ribosylated derivatives and glycosyl conjugates show activity in bioassays, but it is generally believed that they do so after hydrolysis to free bases (Srivastava, 2002).

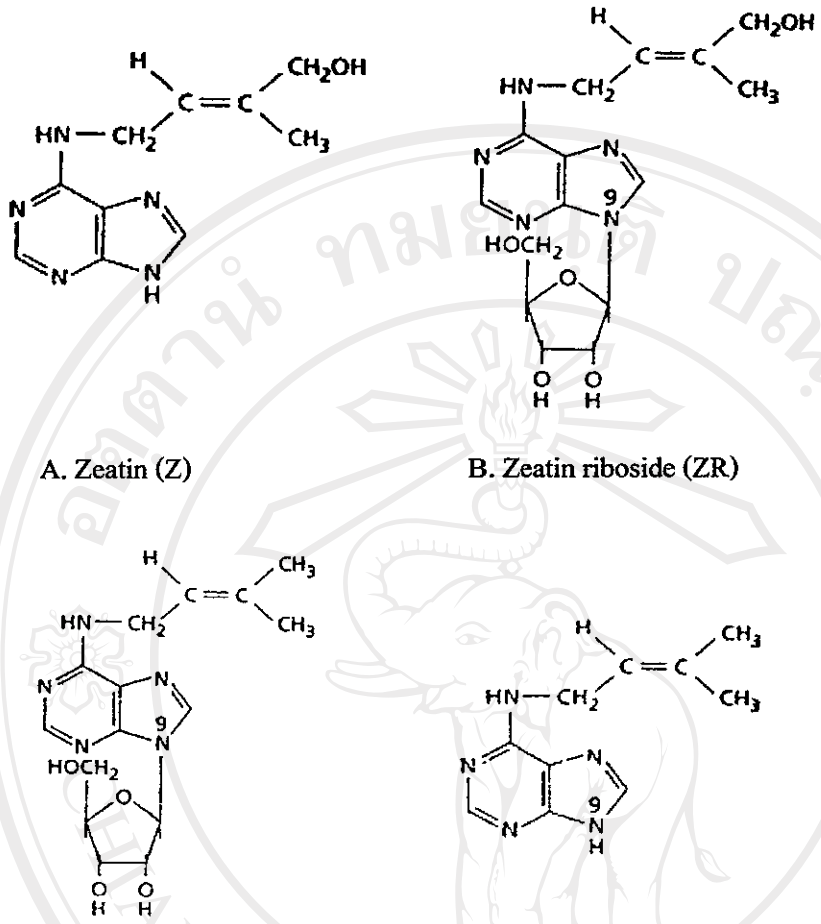
Plant isopentenyltransferases, catalyze the first and rate-limiting steps of CKs biosynthesis, had recently been identified. The first and rate limiting steps of CKs biosynthesis are mainly the isopentenylation of ATP and ADP. The isopentenylated side chain is hydroxylated to form zeatin-type cytokinins (Kakimoto, 2003). Isopentenyl-adenosine (i-Ado) is believed to convert to trans-zeatin *via* isopentenyladenine (i-Ade) or trans-zeatin riboside (ZR) (Figure 2.3) (McGaw and Burch, 1995). Irreversible deactivation of CKs occurs in two ways. The side chain may be cleaved by cytokinin oxidase, an enzyme is induced by high concentrations of CK in plant tissues (Houba-Herin *et al.*, 1999; Srivastava, 2002) and CKs dehydrogenases (Houba-Herin *et al.*, 1999). There are some reports that benzyladenine and kinetin occur naturally. Some synthetic compounds, which inhibit CK promotion of cell division in tissue/cell culture, probably as competitive inhibitors, are known (Srivastava, 2002). Therefore, the levels of active CKs in plants are also expected to be regulated by the rates of biosynthesis, inter-conversion, transport, and degradation (Kakimoto, 2003).

CKs are synthesized at various meristematic sites in the plant, including shoot apex, young leaves, cambial region, and root apices (Srivastava, 2002; Emery *et al.*, 2000). Roots are

known as the major site of CK biosynthesis, but leaves can be additional sites of production in some circumstances (Bernier, *et al.*, 1993). Furthermore, CKs synthesized in the roots are transported upward into the shoot *via* the xylem stream (Srivastava, 2002; Baker, 2000). In *Ricinus*, Baker (2000) determined by GC-MS and reported that ZR, predominant CKs, transports from roots via xylem, whereas Z is a major transported form in phloem. Similarly, increasing CKs level was also found in phloem sap of higher plants (Hoad 1995; Macháckova and Krekule, 2002). From these reasons, the mature leaves could also be a possible source of CKs (Hoad, 1995).

Balla *et al.* (2002) indicated that de novo synthesis of CKs in the buds themselves. In pea, the uptake of the exogenous CK ([^{3}H]Z) reached its peak between the 6 and 8 h after the release from apical dominance. The CK analyses of both short-term and long-term bud cultures revealed the increase of free CKs and their glucosides.

Environmental factors also affect CK levels, which are generally positively correlated with levels of mineral nutrients, especially nitrogenous nutrients (Takei *et al.*, 2001; Sakakibara and Takei, 2002). In litchi, O'Hare (2004) indicated that there was an increase in a rate of root growth with temperature, as well as an increase in CKs just prior to bud-break. Thus, it was possible that such a mechanism might exist in litchi, with root temperature affecting the rate of CKs synthesis. In addition, The effects of low temperature (13°C) on 'Tommy Atkins' mango were indicated that Z/ZR concentrations in terminal buds, bark and wood were greater than at warm temperature (25°C) during days 13-29 of treatment (Naphrom *et al.*, 2004; Naphrom, 2004). Furthermore, mango trees at cool winter, CK-like also increased in xylem sap during early floral induction (Chen, 1987).



A. Zeatin (Z)

B. Zeatin riboside (ZR)

C. Isopentenyladenosine (i-Ado)

D. Isopentenyladenine (i-Ade)

Figure 2.3 Structures of zeatin (A), zeatin riboside (B), isopentenyladenosine (C) and isopentenyladenine (D)

Changes in CK levels in association with plant development have been reported (Dewitte *et al.*, 1999; Howell *et al.*, 2003). Such CKs promoted flower bud differentiation in fruit species, for example, litchi (Chen, 1990, 1991), longan (Chen *et al.*, 1997; Hegele *et al.*, 2004), Japanese pear (Ito *et al.*, 2001) and mango (Naphrom, 2004; Naphrom *et al.*, 2004).

Dewitte *et al.* (1999) studied on distribution in tobacco (*Nicotina tabacum* L.) shoot apices in distinct phases of development using immunocytochemistry and quantitative tandem mass spectrometry. They concluded that during organ formation (e.g. leaves and flowers), was characterized by enhanced CK content. In contrast, during this transition phase, it was found that

three folds of cytokinin ribosides (i-Ade, ZR and dihydrozeatin) decrease in the prefloral transition apices, which showed no organogenesis.

Furthermore, in general, CKs play a major role in the regulation of many processes associated with the supply of nutrients to growing tissues (Roitsch and Ehneß, 2000). It had only few reports show an effect of CKs on the expression of genes of known function, such as nitrate reductase (Lu *et al.*, 1990) extracellular invertase and a hexose transporter (Ehneß and Roitsch, 1997). In addition, Chen (1991) studied on CKs in bud of 'Hey yeh' litchi by using HPLC in combination with *Amaranthus* bioassay and GCMS. Low CK concentrations in 'Heh yeh' litchi were found during bud dormancy and the buds did not respond to exogenous CK application, whereas an increase of CK activity was observed in the buds during flower bud differentiation. Similar to 'Daw' longan, low levels of endogenous CKs in roots during floral induction were also found (Kiatsakun, 2004). Moreover, several physiologists indicated that zeatin increased during the induction period in the off year. They also suggested that an increase in CKs during the induction period possibly had a positive effect on floral formation (Chen, 1987, 1990; Hegele, *et al.*, 2004). Furthermore, Qiu *et al.* (2001) reported the effects of 0.4 $g.L^{-1}$ ethephon in 'Shixia' longan trees. The result was showed that ethephon increased CK and ABA concentrations in buds accompanied with flower bud formation were induced.

According to the researches in litchi of Thailand, Piromya *et al.* (1998) determined on CK-like 'Hong Huay' litchi trees grown in Doi Pui orchard, Chiang Mai by using soybean hypocotyls callus bioassay. The result was showed that flower initiation started from December 16 by determining anatomically technique and CK-like substances increased at 2 and 4 weeks after flower initiation. Furthermore, Naphrom *et al.* (2001) reported that low CK-like concentrations were found during 6-8 weeks prior to flowering of 'Hong Huay' litchi trees grown in Doi Pui orchard, Chiang Mai. The CK-like concentrations increased at four weeks to reach a maximum 2 weeks prior to flowering. Whereas, Lekpaijitr (1999) revealed that low concentrations of CK-like substances were found at week 8^{th} prior to leaf flushing and remained constant until 4 weeks then increased during week 2^{nd} to leaf flushing occurred.

2) Auxins

The most common naturally occurring form in higher plants of auxin is indole-3-acetic acid (IAA) (Figure 2.4). 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, indole-3-glyceerol phosphate and indole, seem 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).

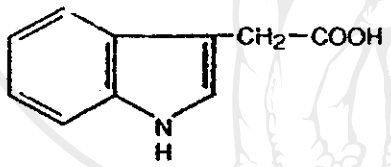


Figure 2.4 Structure of IAA

Most IAA in higher plants occurs in a conjugated form so that free IAA (acid form) levels are low. Conjugate IAA is sometimes referred to as "bound" IAA (or "slow release" form of IAA) as opposed to "free" IAA. Conjugates are inactive form, which hydrolyzed by the plant tissue, and it is the free IAA that gives the activity. The polar transport of IAA in shoots occurs predominantly in a basipetal direction, i.e., from shoot tip toward root. The movement in roots is also basipetal, i.e., from the root tip toward the root-shoot junction (the base of the root). Available data suggest that in shoots, parenchyma cells closed to or associated with the vascular cylinder (bundle sheath cells) are the sites of translocation. Cortical and epidermal cells in root seem to be the primary site for basipetal translocation (Srivastava, 2002).

Endogenous IAA levels are the highest in young tissues, shoot tips, young buds and leaves, young fruit and immature seeds. In contrast, they are usually much lower in older, mature tissues. It is reasonable to assume that IAA is synthesized in young or mature leaves and transported with photoassimilates via phloem pathway to sink tissues such as meristems (Baker, 2000; Srivastava, 2002).

For IAA polar transport, cold temperature might inhibit IAA polar transport out of the shoot tip in pea (Morris, 1979), mango (Naphrom, 2004; Naphrom *et al.*, 2004) and the subtropical trees (Davenport and Nunez-Elisea, 1997). Moreover, Naphrom (2004) studied with 'Tommy Atkins' potted mango trees by keeping under cool temperature (13°C). The results showed that flowering occurred within three months by which IAA level decreased after cool treatment in all plant tissues except in terminal buds where only a little effect could be found. In addition, Li and Bangerth (1999) indicated that high IAA concentration was found in vascular tissue of trees held in warm conditions and it might block IAA transport out of leaves as a result of autoinhibition at the junction of petiole and stem. Furthermore, a reduction in auxin concentration by decapitation increased CK concentration in the xylem in bean (Bangerth, 1994), as well as, interruption of polar IAA transport leaded to a strong increase in root derived CKs (Bangerth *et al.*, 2000).

Several studies on changes in auxin levels concerning with plant development have been reported. Chen (1990) studied on endogenous IAA in the shoot tip diffusates of 'Heh yeh' litchi by HPLC and GC. It was found that diffusible IAA maintained a constant level during the five growth stages included leaf expansion, dormant bud, thirty days before flower bud formation, flower bud formation and full bloom. In 'Daw' longan plants treated with $KClO_3$, Kiatsakun, (2004) reported that auxin-like substances in roots were significantly higher than untreated plants at the second week after treatments, accompanied with inflorescence appearance at day 18 of treatment. In contrast, Hegele *et al.* (2004) investigated endogenous IAA in terminal buds of longan trees treated with $KClO_3$, which grown in Maejo University orchard, Chiang Mai by using radio-immunoassay (RIA). Consistently low auxin values could be detected which visible flower occurred within 17 days of treatment. Furthermore, Pichakum *et al.* (2003) showed that 'Daw' longan trees also grown in Maejo University orchard during 8 weeks before anthesis, level of IAA like substance in terminal shoot tended to increase slowly with development of terminal shoots.

3) Gibberellins (GAs)

GAs are a large class of cyclic diterpenes, now numbering over 110 derivatives defined by their structure. GAs occur in all vascular plants as well as in many fungi. The precursor of GA_1 in higher plants is GA_{20}. Although GA_1 appears to be the primary active GA in stem growth for most species (Davies, 1995; Taiz and Zeiger, 1998), a few GAs have biological activity in other species or tissues. Such as GA_3 is relative rare in higher plants but appears to be able to substitute for GA_1 in most bioassays (Figure 2.5). GA_4 is also as active as GA_1 in some species. Only certain GAs, notably GA_1 and GA_4, are responsible for the effects in plants but the others are precursors or metabolites (Taiz and Zeiger, 1998).

GA biosynthesis occurs in three stages in three distinct subcellular locations. First stage, geranylgeranyl diphosphate is converted in a two-step process to *ent*-kaurene and these steps occur in plastid. Secondly, *ent*-kaurene is translocated to the cytoplasm, where it is progressively oxidized and hydroxylated to yield GA_{12}-aldehyde, which is the first gibberellin formed in all plants and thus is the precursor of all the other gbberellins. All enzyemes involved are monooxygenases that utilize cytochrome P450 in their reactions. These P450 monooxygenases are localized on the endoplasmic reticulum, where it is converted to GA_{12} (Hedden and Kamiya, 1997). In the third stage, GA_{12}-aldehyde is further oxidized, hydroxylated, and, in some cases, desaturated by soluble enzymes in the cytosol to yield the bioactive C_{19}-GAs (Taiz and Zeiger, 1998).

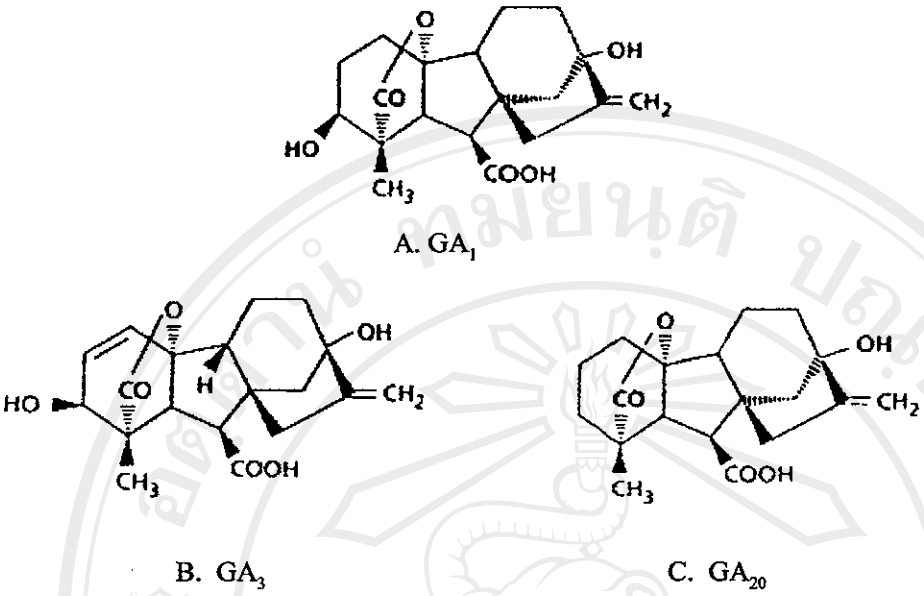


Figure 2.5 Structures of GA_1 (A), GA_3 (B) and GA_{20} (C)

GA homeostasis is maintained not only by the control of synthesis but also by the inactivation of bioactive GAs by conjugation and/or irreversible deactivation *via* 2ß-hydroxylation. GAs occur primarily in the young, actively growing buds, leaves, and upper internodes. These tissues also appear to be the sites of GA synthesis (Sherriff *et al.*, 1994). The GAs synthesized in the shoot can be transported to the rest of the plant *via* the phloem (Srivastava, 2002).

Effect of low temperature on GA level in plants, the low temperature decreased endogenous GA level and then induced flowering of citrus (Goldschmidt *et al.*, 1997). In contrast in pea, low temperature (3-5°C) induced a 5-10-fold enhancement of ent-kaurene, hence potentially GA, biosynthesis in seedlings of two dwarf pea cultivars, but not in the tall cultivars. However, the lack of an increase in growth rate in the cold-treated dwarfs indicated that endogenous GA biosynthesis remained blocked at some point beyond ent-kaurene in the biosynthetic pathway (Thomas *et al.*, 1991). In *Campanula isophylla*, Jensen *et al.* (1996) studied on effect of day/night temperature regimes on stem elongation and on the content of endogenous GAs in vegetatively propagated plant by using GC-MS. The result was showed that temperature regimes of 21/15°C stimulate elongation growth were accompanied by an increase in the level of

GA_1, GA_{19} and GA_{44}, whereas plant grown under 15/21°C reduced stem elongation growth with an increased level of GA_{97}.

GAs are involved in several important biochemical and morphogenetic responses. One very common response is the promotion of elongation in certain types of plants, such as dwarf rosette plants. Other physiological effects of GAs in vascular plants include changes in juvenility, flowering and floral development, promotion of fruit set, fruit growth, and seed germination (Taiz and Zeiger, 1998; Srivastava, 2002).

For GAs concerning flowering in fruit trees, induction of flowering were observed when endogenous GAs decreased such as in citrus (Goldschmidt *et al.*, 1997), litchi (Chen, 1990; Naphrom *et al.*, 2001), longan (Boonplod, 1994; Hegele *et al.*, 2004), mango (Pongsomboon *et al.*, 1997; Naphrom *et al.*, 2004) and other species (Ulger *et al.*, 2004). Koshita *et al.* (1999) investigated endogenous GAs of satsuma mandarin (*Citrus unshiu* Marc.) by using ELISA technique. It was suggested that $GA_{1/3}$ contents in leaves was considerably higher than $GA_{4/7}$. Not only exogenous GA but also endogenous $GA_{1/3}$ reduced flower bud formation in October. Furthermore, some GAs derivatives found to promote flower formation in citrus (Goldschmidt *et al.*, 1997; Koshita *et al.*, 1999) and olive (Ulger *et al.*, 2004). Similar to 'Tommy Atkins' mango, Naphrom (2004) reported that after expose mango trees to cool temperature (13°C) for weeks, low GA concentrations were found in terminal bud. In addition, Qiu *et al.* (2001) reported that in longan trees treated with ethephon application GA concentrations in buds decreased accompanied with flower bud formation were induced. In litchi, Chen (1990) reported that in 'Heh yeh' litchi, high endogenous GAs were found in xylem sap at stage of leaf expansion. Low level of GA concentrations in xylem sap were observed at a low level at 30 days before and through out the stage of flower bud formation.

In Thailand, Naphrom *et al.* (2001) studied on endogenous GA-like substances in shoot apex of. 'Hong Huay' litchi trees grown in Doi Pui orchard, Chiang Mai during December 1997-January 1998 by using rice secondary leaf sheath bioassay. The results were showed that GA-like concentrations in terminal shoots decreased from weeks 4 to weeks 2 prior to flowering which it level at weeks 2 prior to flowering was below detectable levels at flower emergence (Naphrom *et al.*, 2001). Similar to 'Daw' longan, Boonplod (1994) revealed that activity of GA-like substances in week 6 prior to flowering was high and constant until weeks 3, after that it

decreased to minimum at the week of flowering. Moonfoui (2001) also reported that in 'Daw' longan trees, the amount of GA-like substances decreased before flowering whereas it increased before leaf flushing.

Recently, it was shown that normal levels of IAA are required to maintain normal level of bioactive GAs (GA_1) in elongating pea stems (Ross *et al.*, 2000; Ross and O'Neill, 2001). Promotion of GA_1 biosynthesis by IAA was confirmed using a traditional technique of applying IAA (in lanolin paste) to a cut stump of decapitated plants, and could employ to replenish stem IAA content. This simple procedure completely retained the stem's ability to synthesize and accumulate GA_1.

4) Abscisic acid (ABA)

ABA is a 15-carbon terpenoid compound derived from the terminal portion of carotenoids. It is synthesized in almost all cells that contain plastids and is transported *via* both the xylem and the phloem. ABA produces in roots and mature leaves (Hartung *et al.*, 2002). Moreover, the level of ABA fluctuates dramatically in response to developmental and environmental changes such as water stress (Taiz and Zeiger, 1998). ABA also increases during cold acclimation in a number of plants such as in mango tree (Naphrom, 2004). Moreover, ABA might act through the ethylene signal transduction pathway to inhibit root elongation, and that ethylene and ABA compete with each other to activate that pathway (Ross and O'Neill, 2001). However, ABA is very effective in causing stomatal closure, and its accumulation in stressed leaves plays an important role in the reduction of water loss by transpiration under water stress condition (Taiz and Zeiger, 1998).

ABA plays major roles in seed and bud dormancy. It has little known about the role of ABA in buds, ABA is one of the inhibitors that accumulates in dormant buds. It also antagonizes the action of GAs by suppressing the synthesis of œ–amylase by barley aleurone layers (Taiz and Zeiger, 1998). Furthermore, in longan, Pichakum *et al.* (2003) reported that ABA like substance remained at steady low levels at the age of terminal shoot during 8 weeks before anthesis.

5) Ethylene

Ethylene, a simple organic molecule, Ethylene is synthesized in most organs of flowering plants from methionine *via* a cyclic pathway. Senescing tissues or ripening fruits produce more ethylene than young or mature tissues do. The precursor of ethylene *in vivo* is the amino acid methionine, which is converted to *S*-adenosylmethionine, 1-aminocyclopropane-1-carboxylic acid, and ethylene. Ethylene biosynthesis is triggered by various developmental processes, by auxins, and by environmental stresses (Taiz and Zeiger, 1998; Srivastava, 2002).

Ethylene regulates fruit ripening and other processes associated with leaf and flower senescence, leaf and fruit abscission (Taiz and Zeiger, 1998; Srivastava, 2002). However, it induces flowering in pineapple, and therefore being used commercially in pineapple for regular flowering and fruit set. Flowering of other species, such as mango, is also initiated by ethylene (Taiz and Zeiger, 1998). In litchi, Olesen *et al.* (1999) also reported that ethephon concentration at 1000 to 3000 mg l^{-1} plus 0.5 % urea caused defoliation of late autumnal vegetative flush when applied in May in subtropical Australia. After that, within a few weeks new buds under damaged shoots become active and flowered if the weather remained cool enough.

Furthermore, Thonglem (2002) studied on changes in ethylene concentration in intercellular space in shoot of 'Daw' longan, 'Hong Huay' litchi and 'Toon Kloaw' marian plum during 8 weeks before flowering. It revealed that in longan ethylene concentration tended to reduce from 8^{th} to 4^{th} week prior to flowering and then increased dramatically in the 2^{nd} week before flowering. Whereas ethylene concentrations in litchi and marian plum tended to increase from 8^{th} week prior flowering until the flowering periods.

2.8 Effect of agricultural practices

2.8.1 Girdling or cincturing

It is one of the practical techniques to control the timing of flushes and improve flowering in litchi as shown in Mauritius (Ramburn, 2001), China (Li and Xiao, 2001), Australia (Menzel, 2002a, 2002b) and Thailand (Manochai and Jarassamrit, 2000; Sethpakdee, 2002; Sruamsiri *et al.*, 2003). It will interrupt the phloem translocation from leaves to roots or to lower part of girdled wound.

Chen and Huang (2001) suggested that in China, spiral girdling on litchi was useful for multi-propose reasons. These reasons were included speeding up a maturation of autumn flushes, inhibiting winter flushing in favor of flower initiation, increasing percentage of pistillate flowers, overcoming excessive fruit drop and improving fruit size and quality. Moreover, for floral induction, girdling should be made at a stage between the turning green of leaves and flower initiation during winter. According to fruit setting, it should be immediately girdled after blooming.

Similar to litchi in northern part of Thailand, according to reduce new leaf flushing prior to flowering and promote flowering. Sruamsiri *et al.* (2003) suggested that the suitable stage of girdling in 'Hong Huay' lichi could be done at the beginning of mature leaf stage of the 2nd flushed shoot during September to October. Litchi flowering occurs profusely when the temperature is cool enough.

However, Kaosampan *et al.* (2003) indicated that branch girdling could not promote a number of flowering trees and flower density of litchi whenever weather was cool enough for off-season flowering at upper hills in northern part of Thailand. In addition to, Manochai *et al.* (2003) stressed that trunk girdling could be used as a tool to induce flowering with uniform and rapid flowering in 'Petsakorn-twai' longan trees.

2.8.2 Pruning

Normally, litchi trees are pruned in summer after harvesting, to control tree size (Goren and Gazit, 1993; Menzel, 2002a) and promote flowering intensity and yield in the litchi trees (Menzel, 2002a; Stern *et al.*, 2003).

Moreover, root pruning during winter is also adopted for stimulating floral initiation in litchi (Chen and Huang, 2001). Similar to longkong (*Lansium domestica* Corr.) grown in cement pipe with 50% shade and in the field, Ruaenngarn *et al.* (2002) suggested that root pruning at 25% of canopy area could induce flower bud from both of grown conditions. In addition, all levels of root pruning (12.5, 25 and 50% of the canopy area) could induce flowering better than no root pruning.

2.8.3 Chemical application

Many chemical substances and growth retardants have been studied for promoting flowering in litchi trees, but none consistently effect is achieved. Stern (1992) for example, applied litchi with Alar (85% of 2,2-dimethylhydrazide) and Cyclocel (40% of 2,chloroethyl-trimethyl-ammonium chloride) at 0.5 % with no success. Olesen *et al.* (1999) treated litchi with ethephon at 1,000-3,000 $mg.l^{-1}$ plus 0.5% urea, it caused defoliation of the late autumnal vegetative flush when applied in May in subtropical Australia. New buds emerged below the damaged shoots within a few weeks, and flowered if the weather remained cool enough for flower initiation. Paclobutrazol alone, and combined with ethephon (Chaitrakulsub *et al.*, 1992; Ramburn, 2001) or combined with $KClO_3$ (Sruamsiri and Manochai, 2001; Sritontip *et al.*, 2003; Sruamsiri *et al.*, 2003) were also tested, but without any positive as well. Ngernsri (2003) also revealed that the treatment of 5,000 and 10,000 ppm of $KClO_3$ concentrations had no effect on new shoot development, TNC, CK-like substances and flowering in 'Chakrapat' cultivar.

Concerning nutrient application in litchi trees, Wanichanukul (1990) reported that 'Hong Huay' responded more sensitive to monopotassium phosphate (0-52-34) for flowering than 'O' Hae'. Foliar spray at the end of rainy reason and during young expanding leaf stage, three times, with 3,750-5,000 ppm of 0-52-34 was proved to be suitable to promote a better flowering. Plant with high nitrogen contents in leaves tended to develop a vegetative flushing, and reduced flowering (Menzel and Simpson, 1991; Li *et al.* (2001).

2.8.4 Water management

Normally, litchi flowering is favored by a dry period before and during floral initiation (Stern and Gazit, 2003). Stern *et al.* (2003) conducted a trial with four autumnal irrigation regimes 100, 50, 25 and 0% of the recommended irrigation rate. All three water stress treatments restricted autumnal shoot growth, and significantly increased the flowering intensity and yield. On the other hand, moderate water stress is sufficient to induce fruit bud differentiation without any visible damage to the trees. Moreover, dihydrozeatin-riboside and ZR concentrations in the xylem-sap increased with decreasing irrigation level, down to the 25 % irrigation level (Stern *et al.*, 2003).

2.8.5 Young leaves destruction

In litchi, removal of young flush releases the buds on the proximal mature shoot from its apical inhibition. These active buds respond to the inductive condition and are transformed into floral buds (Batten and McConchie, 1995; Rankunta, 1997). Small size of terminal buds (less than 3 mm) that start to grow under noninductive conditions will form normal inflorescences if they are transfer to inductive conditions (Batten and McConchie, 1995). Similar to mango, Nunez-Elisea and Davenport (1995) reported that shoot tip at the leaf age less than 2 weeks could not flower whether it received suitable low temperature for floral induction. Moreover, it had been reported that floral stimulus signal transferred from mature leaves *via* the phloem to the apex (Bernier *et al,* 1993) such as in longan (Sruamsiri *et al.*, 2003) and mango (Nunez-Elisea and Davenport, 1992; Nunez-Elisea *et al.*, 1996). However, Hegele *et al.* (2004) and Naphrom (2004) revealed that endogenous IAA exported from young leaf was greater than old leaf. IAA can also be synthesized in leaves and transported with photoassimilates *via* phloem to the shoot meristem (Davies, 1995; Baker, 2000; Srivastava, 2002). Therefore, it may be possible that IAA translocates from the young leaves to the terminal shoots may act as inhibitory hormone to floral induction.

2.9 Interaction of environment, plant development stage and practice on flowering and possible role of plant hormone

Normally, it is known that litchi flowering is favored by a dry and low temperature period before and during floral induction, so that promotion of autumnal water stress and water management are the techniques being used in litchi orchards in order to induce profuse flowering (Menzel and Simpson, 1994; Gazit, 2001; Stern and Gazit, 2003). However, the main factor is low temperature which it affects to irregular bearing in some years whenever the temperature during winter is not cool enough. Therefore the interaction among external factors such as drought and low temperature require for promote flowering as well as endogenous factors. Several physiologists suggest that high cytokinin and low auxin may be required as a prerequisite for floral induction, so that the techniques which reduces endogenous auxin and enhance cytokinin synthesis should be effective to promote flowering. In addition, pruning techniques after harvesting is also farmers used for recovering healthy supply of the tree, as well as girdling

prior to preseasonal flowering for stop new leaves flush during September-December. Whenever a new shoot with leaf flush occurs short before the flowering period, high concentrations of auxin in the young leaves may inhibit flower bud formation. Therefore, shoot tip pruning can solve the problem of pre-seasonal flowering and increase homogeneity of in-season flowering. Therefore, understanding of physiology of flowering should be necessary to improve this irregular bearing problem.

Recently, several researchers proposed that plant development, physiology and metabolism, especially flowering are regulated by input from a number of signaling/response pathways. These involve in response to phytohormones, environmental stimuli and metabolites such as carbohydrates (Bernier, 1988; Nunez-Elisea *et al.*, 1990; Menzel and Simpson, 1994; Machácková *et al.*, 1996; Levy and Dean, 1998; Gibson, 2000). Similarly, these studies also proposed flowering in litchi may be manipulated by multifactors, so that these studies are conducted to understand the correlation between the balance of endogenous hormones and assimilative substances in plant tissue with the floral induction in litchi.