Chapter 2

Review of Literature

1. Origin and distribution

Longan (*Dimocarpus longa*n Lour.) is belongs to the Sapindaceae family similar to lychee (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* Linn.) and pulasan (*Nephelium mutabile* Labill.), but differ in fruit morphology and ecology. It is believed to have originated in the mountain chain that stretches from Myanmar through southern China and possibly down to lowlands of south-west India and SriLanka (Tindall, 1994). Wild trees have been found dominating the tropical or monsoon rainforests in central and south-west Hainan and in the west and south-west of Yunnan (Huang *et al*., 2005). Longan is mainly grown in southern China, Taiwan and Thailand. Smaller growing areas are found in Vietnam, Cambodia, Laos, Queensland (Australia), Indonesia and Florida (United States) (Anupant and Sukhvibul, 2003). It is believed that longan were brought to Thailand from China in the late 1800s, although native longan cultivars that produce low-quality fruit were also found growing in northern Thailand over 200 years ago (Angasith *et al*., 1999; Subhadrabandhu and Yapwattanaphun, 2001).

2. Botany of longan

Longan is an evergreen tree that can grow up to 20 m in height, and has a spreading or erect habit, depending on the cultivar. The trunk is brittle, with the branches having a corky bark that splits and peels. The compound leaves are alternate and pinnate with 3 – 5 pairs of leaflets, 3 - 6 cm in width, 7 – 15 cm in length and dark glossy green on the adaxial and paler green on the abaxial. The inflorescences

are compound dichasia borne on terminal shoots, 8 – 60 cm in length and with many branches (Subhadrabandhu and Stern, 2005).

Flowers are normally small and yellowish brown, on a lobed calyx 2-5 × 1-3 mm. They have five white and woolly to glabrous petals. There are three types of flowers. The male flowers have about 6 – 8 hairy stamens arranged in a single row on a light-brown disc with two-lobed anther. The female flowers contain bicarpellate hairy ovaries with a bilobed stigma and sterile anthers that have short filaments. The perfect flowers or hermaphrodite flowers which have eight stamens with sessile filaments and produce viable pollen. Fruits normally take 120-150 days from full bloom to mature. The fruit exocarp is thin, but tough and leathery, and changes from greenish yellow to yellowish brown with advancing to maturity. They are juicy to very crisp and tasteless to sweet and aromatic. There are only one seed in fruit. Seed shape is globular and shiny, brown to dark brown (Subhadrabandhu and Stern, 2005).

3. **Flowering process**

Important centre of floral transition is a group of stem cells called the shoot apical meristem (SAM). Changes in organogenesis occurred through phase transitions at the meristem. The vegetative phase is characterized by juvenile and then a competent adult stage prior to the floral transition. However, the process of flower formation can be split into several discrete steps: the first of these is the induction of flowering that is the decision to switch from the vegetative to the reproductive phase. The second step is evocation, transition from the vegetative shoot meristem (VM) to the inflorescence meristem (IM), with the associated generation of the primodia of flowers, lateral organs, and the cauline leaves. The third step is the actual formation of the flower. As fourth and last step, the functional phase of the flowering process begins, when the flower organ have gained their characteristic forms and functions. During this functional phase the maturation of the reproductive organs, pollination and fertilization take place (Westhoff *et al.*, 1998).

4. The control of flowering

The three models for controlling the flowering time have been proposed. The first was that the florigen, a flowering-promoting hormone, was produced in mature leaves under favorable conditions such as photoperiods, low temperature and transported to the shoot apex via the phloem (Evans, 1971). But the chemical nature of this stimulus is still unknown. The nutrient diversion hypothesis was the second model which proposed that inductive treatment result in an increasion in the amount of assimilates moving to the shoot apical meristem, which is relatively deprived of nutrients during reproductive development or must receive a higher level of assimilates for gene expression than required for vegetative development. The class of chemicals, climatic conditions and management that mobilize nutrients at shoot apical meristematic tissues or suppress competitive sinks for assimilates at times appropriate for floral initiation (Sachs, 1977). The last model is multifactorial control model, which proposed that a number of promoters and inhibitors, including phytohormones and assimilates are involved in controlling the developmental transition (Bernier, 1988). According to this model, many molecules promote flowering, but none is sufficient to trigger flowering universally. Flowering can only occur when the limiting factors are present at the apex in appropriate concentrations and at the right times (Levy and Dean, 1998). One model for the multifactorial control of flowering has been developed on the basis of experiments utilizing the long-day plant, *Sinapis alba* (white mustard). This model postulates that flowering is regulated by severals molecules, including plant hormones and other common metabolites. Those researchers and others have identified flower-promoting roles for GA, cytokinin, sucrose, and polyamines in annual mustards such as *S.alba* and *Arabidopsis*.

5. Factors affecting flowering

The flowering of plants is a complicated developmental process that involves a series of morphological and physiological stages under the control of a number of external signals and internal factors. Among all factors that control plant flowering, photoperiod and plant hormones have been studied in great detail.

5.1 External or environmental factors

Under natural growth conditions, flower formation usually commences when the plant reaches a certain age. The age is, within certain limits, genetically fixed in a species-specific way. This is seldom spontaneous; usually it is induced by external factors such as temperature and light which are very important for flower induction (Westhoff *et al*., 1998).

Often, flower formation requires that the plant is exposed to low temperatures, usually between a few degrees above zero and 15 °C. The stimulation of flower development by low temperatures is called vernalization. The site of cold-temperature perception of vernalization is the shoot apex and actively dividing cells and the duration of chilling temperature treatment required for the maximal acceleration of flowering can range, according to species, from four days to three months. Vernalization can often be reversed if followed immediately by a period of high temperature. For tropical fruit, mango required about 15 °C for 30 days to promote inflorescence morphogenesis (Batten and McConchie, 1995; Nunez-Elisea and Davenport, 1995; Nunez-Elisea *et al*., 1996). For subtropical fruit, litchi flowering also induced by cold temperature, flowering was very weak when day shoot temperatures and root temperatures exceeded 20 °C. In litchi cv. Tai So, panicle were visible from 3-7 weeks after transfering to low temperatures with emergence earliest at 20 °C during the day and 12.5 °C during the night (Menzel *et al*., 1989). Longan grows well in the tropics but requires a prominent change of seasons for satisfactory flowering. A short duration (2-3 months) but cool (mean temperature 15-22 °C) in cold season induces flowering (Wong and Ketsa, 1991). However, they are sensitive to frost and are killed or severely injured by prolonged temperatures below freezing (Menzel *et al.*, 1995).

The second important external factor that can induce flowering is light. In many cases, it is not the intensity, but the duration of the day and night period or photoperiod that are important. Depending on the light necessary for flower induction, three main group of flowering plants can be distinguished: short-day plants (SDP), plant in which flowering is promoted when they are grown under long-night/short-day condition; long-day plants (LDP), plants in which flowering is promoted in short-night/long-day conditions and day-neutral plants (DNP) which is not affected by photoperiod (Thomas and Vince-Prue, 1997). Photoperiodic induction occurs in the leaves with photoreceptors, which initiate signals that translocated a considerable distance to the SAM. The initiated flowering signals can be transmitted from an induced to a non-induced plant of the same species by grafting and, in some case, transmission is possible between species, even if these have different photoperiodic requirement: e.g. the induction of flowering may be stimulated in a SDP (non-induced) by transmission from a LDP (induced), and transmission may occur between day-neutral and day-sensitive plants. These results are difficult to interpret in terms of a single stimulus which promote flowering. It is clear, however, from both physiological and genetic studies, that the induction of flowering is a multifactorial process, with both stimulatory and inhibitory processes interacting in a complex net work to regulate flowering. However, longan is the day-neutral plant but light has direct effects on photosynthesis pathway and accumulation of carbohydrates (Menzel *et al*., 2000).

Besides those, flowering can also be induced by others factors such as nutrient deficiency, drought (water stress) and cultural practices. Litchi flowered after a period of vegetative dormancy induced by leaf water stress only if they were subsequently transferred to low temperatures (Menzel *et al*., 1989). Angasith *et al*. (1999) suggested that longan should be pruned, fertilized (high N) and irrigated immediately after harvesting to induce new leaf growth. Soil moisture should be lowered and nitrogen fertilizer applications uphold until flowering or two months before then to allow the mature flush to “rest”. Pre-flowering fertilizers should contain high P and K. After flowering and a month prior to harvest, fertilizers with high N and P and high K were recommended. It was reported in litchi that cincturing of branches and stems could induce dormancy and resulted in better flowering, fruiting and yield. However,

the results did not consistent. In longan, the easy-to-flower cultivar 'Phetsakorn' could be induced to produce early and uniform flowering by cincturing of branches or stems (Subhadrabandhu and Yapwattanaphun, 2000).

5.2 Internal or endogenous factors

Plant nutrition is proposed to be one of internal factors, strawberry cv. Toyonoka was placed under flower inductive conditions (low temperature and short photoperiod) and ^{15}N were applied as a solution of KNO_3 on day 16. The result showed that more N was allocated to shoot apex, crown and root of induced plants (Yamasaki *et al*., 2002). But, certain amount of N should be controlled in some plants. In Australia, the most effective method to prevent vegetative growth in litchi is to maintain leaf N content at 1.75-1.85% by applying nitrogen fertilizer only after panicle emergence and fruit set (Zee *et al*., 1999). Nitrogen sensing appears to regulate a variety of physiological and developmental processes in plants. The mechanism is nitrate activated transcription of genes that are involved in nitrate transport (Coruzzi and Zhou, 2001). Expression of nitrate reductase (NR) and nitrite reductase (NiR) genes is induced by nitrate. Nitrate is known to regulate via the intermediate of the glutamine and glutamate ratio, the phosphorylation and activity of phosphoenolpyruvate carboxylase (PEPase), sucrose phosphate synthase (SPS) and NR enzyme that controls the distribution of photosynthetic carbon between the synthesis of sucrose and amino acids (Limami and Ameziane, 2001).

Sugars have been implicated in control of many plant processes, the molecular mechanisms by which sugars act remain largely unknown. Although glucose or sucrose may act directly as a signaling molecule in some sugar-response pathways, other pathways may sense the level of a different sugar or sugar metabolites (Gibson, 2000). The evidence that sucrose may function in long-distance signaling during floral induction comes from studies of *Sinapis alba*, a long-day plant in mustard family. After induction of flowering by either a single long day or by a displaced short day, the concentration of sucrose in the phloem reaching the shoot apex increases rapidly within one hour of the photo extension for a long day treatment and transiently. This pulse of sucrose translocation has just before the increase in cell

division that normally is observed in the shoot apical meristem during floral evocation (Bernier *et al*., 1993). The *in vitro* culture of plants on vertically placed medium containing 1% (w/v) sucrose in the dark or in the light, partially reduced some of the late-flowering phenotypes mutants. These results supported the positive role of sucrose in floral transition in *Arabidopsis* (Roldan *et al*., 1999). It is interesting that the flowering promoting gene LEAFY is induced by sucrose (Smeeken, 2000). However, high level of sucrose in the medium significantly delayed flowering in *Arabidopsis*. High concentration of sucrose (5% w/v) in the medium also delays floral transition of all early and late flowering (Ohta *et al*., 2001). These results lead to the conclusion that sugar in the medium inhibits floral transition in at least two different ways. The promoting and inhibiting effects of sugar on flowering depends on the concentration and time of addition of sugar and the genetic background of plants (Bernier *et al*., 1993). In litchi, there is a strong correlation between flowering and starch content under different temperature regimes (Menzel *et al*., 1989). However, the effect of girdling on flowering of citrus cannot be explained solely in terms of carbohydrate accumulation (Garcia-Luis *et al*., 1995). Moreover, there does not have an evidence that high starch level as trunk concentration of mango promotes flower initiation at 20/15 $^{\circ}$C and 15/20 $^{\circ}$C day and night temperatures (Whiley *et al*., 1989). In pummelo cv. Tosa Buntan, also found that leaf carbohydrate content could not account for the promotion effect of starches on flower induction and initiation, but their level at the time of flowering seemed to directly relate to the growth and yield (Yamanishi, 1995).

A hypothesis is that those mutants lack starch, which is normally degraded in the dark phase to provide small carbohydrate metabolites that serve as a signal for floral initiation, along with other environmental and developmental cues. The analysis of carbohydrate metabolites and floral induction of the starchless phosphoglucomutase (*pgm*) mutant support this hypothesis. Late-flowering phenotype of the pgm mutant in *Arabidopsis* is due to the impossibility of mobilizing carbohydrate reserves in conditions in which floral induction is not accompanied by increased photosynthesis. Furthermore, the result showed that the late flowering phenotype under short day conditions of the 1-1 phosphoglucoisomerase (*pgi*) mutant can be reversed to wild type by the addition of 1% (w/v) glucose, fructose or sucrose

in the growth medium (Yu *et al*., 2000). Starch mobilization is an essential process in the control of flowering transition in wild type *Arabidopsis* as it is in wild type *Sinapis alba* (Bernier *et al*., 1993).

Sugar sensing is complex in higher plants because their photosynthetic activity, the uptake and assimilation of other nutrients and the distribution of sugars throughout the plants. Sugar responsive genes in representatives of each plant are the first step of glycolysis; the phosphorylation of glucose by hexokinase. Role for hexokinase in sugar regulates expression of gene encoding glyoxylate pathway enzymes and photosynthetic proteins (Taylor, 1997). In maize mesophyll, seven photosynthetic genes were coordinately represent by glucose and fructose (Coruzzi and Bush, 2001). Some experiments suggested that hexokinase played a key role in transducing a hexose dependent signal in the maize protoplast system. Even though, photosynthetic genes are repressed most by acetate and often more strongly by hexoses than sucrose (Koch, 1996).

The carbon balance of the leaf is relation to photosynthesis, respiration, and incorporation or loss of carbon during growth, the comparable balance for nitrogen in terms of import, assimilation, storage and export of specific nitrogenous solutes and the interactive economics of carbon and nitrogen as dictated by C:N ratios of incoming and outgoing solute streams (Bourgeais-Chaillou *et al*., 1992). The interaction between C and N metabolites in higher plant cell is governed by many regulatory factors. The coordinate of C and N metabolism is reflected by the complex interplay between signals involving carbon metabolism, such as sucrose and light, and those associated with nitrogen metabolism, such as nitrate (Lancien *et al*., 1999). C:N sensing mechanism enables plants to activate genes involved in N-assimilation when carbon skeletons are abundant and internal level of organic-N are low, or to halt N- assimilation when levels of photosynthate are low or internal levels of organic-N are high (Gocal *et al*., 2001). Carbon and nitrogen metabolisms are tightly linked and it seems obvious that nitrogen signaling pathways interact with sugar signaling pathways. Such the interaction in turn may be controlled or mediated by phytohormones (Smeeken, 2000). Glucose signaling in plants has been shown to involve complex cross talk with hormone signaling pathways (Ohta *et al*., 2001).

Carbon metabolite was found signal to linked to ethylene, abscissic acid (ABA) and GA response pathways (Coruzzi and Bush, 2001).

Plant hormones have been proposed to involve in flowering of various kinds of plants. There are two kinds of hormones, base on the ability to move across the plasma membrane. Lipophilic hormones bind mainly to receptors in the cytoplasm or nucleus but water-soluble hormones bind to receptors located on the cell surface. In either case, ligand binding alters on the receptor, typically by causing a conformational change. Some receptors can regulate gene expression directly, however, in the vast majority of cases, receptor initiates one or more sequences of biological reactions that connect the stimulus to a cellular response. Typically, the end result of signal transduction pathways is to regulate transcription factors, which in turn regulate gene expression. Auxin, cytokinin and gibberellin should increase assimilate transport towards the kind of treated tissues and may promote or inhibit flowering depending upon the tissues treated and their specificity of action (Sachs, 1977).

Auxin promote elongation growth primarily by increasing the cell wall extensibility. The ability of protons to cause cell wall loosening is mediated by a class of proteins called expansins by breaking hydrogen bonds between the polysaccharide components of the wall. Signal transduction pathways involved in auxin action have implicated cyclic AMP and mitogen-activated protein kinase and possible signaling intermediates in auxin-induced cell division. Other possible auxin signaling intermediated includes Ca^{2+}, intracellular pH and lysophosphatidyl choline. The ratio of auxin to cytokinin determines the differentiation of cultured plant tissues into either roots or buds, high ratios promote roots but low ratios promote buds (Zeiger, 1998).

The gibberellin are a large group of related compounds (more than 110 are known) that, unlike the auxin, are defined by their chemical structure rather than by their biological activity. Gibberellins are most often associated with the promotion of stem growth, and the application of GA to intact plants can induce large increase in plant height. As we will see, however, GA play important roles in a variety of physiological phenomena but unlike auxin biosynthesis, GA biosynthesis is under

strict developmental control, and numerous GA-deficient mutants have been isolated. Mendel's tall/dwarf alleles in peas are a famous example. Such mutants have been useful in elucidating the complex pathways of GA biosynthesis. Endogenous gibberellin influence a wide variety of developmental processes. In addition to stem elongation, gibberellin control various aspects of seed germination, including the loss of dormancy and the mobilization of endosperm. In reproductive development, GA can affect the transition from the juvenile to the mature stage, as well as floral initiation, sex determination, and fruit set. GA can substitute for the long-day or cold requirement for many plants, especially rosette species, that require either long days or low temperatures of flower. GA may thus be a component of the flowering stimulus in some plants, but apparently not in others (Opik and Rolfe, 2005).

Cytokinin activate the expression of genes associated with cell division and differentiation, stress or defense responses and sink/source metabolism. In shoot regeneration, cytokinin most likely sets in motion a cascade of development events not all of which are directly affected by the hormone. Primary signaling events generally occur within a minute. Primary signaling events occur without new protein synthesis while secondary events involve new gene expression and protein synthesis. Cytokinin activation or inactivation can occur at the transcriptional level or other levels such as the post-transcriptional or translational level. Cytokinin is defined by its ability to stimulate cell proliferation in cell culture in the presence of auxin. It has been shown that cytokinin activates the expression of a number of different genes involved in the regulation of the cell cycle at S/G1 phase.

Ethylene is predicted to act as a transcription factor to regulate gene expression (Hooykaas *et al*., 1999). Most of hormones that affect on development are rather secondary effect than primary effect. Therefore, many kinds of proteins affected by hormones are found such as the accumulation of α-amylase in germination seed regulated by GA from embryo (Zeiger, 1998). Similar to the induction of flowering affected by environment, Hirai *et al*. (1994) studied in morning-glory found that the content of two-phenylpropanoids were increased during low temperature and they were identified as 3-o-feruloylquinic acid and dehydrodiconiferyl alcohol-13-o-beta-D-glucoside. The increasing was more rapid in

the cotyledons exposed to high-intensity light before the low temperature. This suggested that the accumulation of these compounds might be correlated to the promoted effect of high-intensity light on the flower induction by low temperature. In *Arabidopsis*, there are four pathways to induce flowering. All pathways are either induced by environment, stage of growth or GA, many down stream genes involve in flower initiation and development.

There is an evidence from studies on *S. alba* that long-distant signaling by cytokinin might play a role in the transition to flowering in response to inductive photoperiod. Inductive photoperiods cause the rapid and transient export of sucrose from the leaves to both the shoot and root meristem. In the root, this sucrose leads to export of cytokinin, primarily zeatin riboside, to the shoot and leaves via the xylem. Subsequently, another cytokinin, isopentenyladenine riboside, moves out of the leaves, and some makes its way to the shoot apex, where its levels increase within 16 hours of induction (Bernier *et al*., 1993). In litchi, the lack of flowering under moisture stress at 30/20 $^{\circ}$C (12.5 $^{\circ}$C root temperature) could not be attributed to low starch reserves and is possibly related to high gibberellin, low cytokinin activity (Menzel *et al*., 1989). On the other hand, cytokinin found not involve in flower induction. The prefloral transition apices of tobacco (*Nicotiana tabacum* L.) detected no free cytokinin bases (zeatin, dihydrozeatin, or iso-pentenyladenine) and also observed a three-fold decrease in the content of cytokinin ribosides (zeatin riboside, dihydrozeatin riboside and iso-pentenyladenosine) during the transition phase (Dewitte *et al*., 1999). In undifferentiated buds of litchi and longan, found generally low in total cytokinin and to increase many fold after flower bud initiation (Chen, 1991; Huang, 1999). In longan, it also was found a large decrease in cytokinin glucocides and an increase in zeatin and zeatin riboside activities in bud during floral initiation (Chen *et al*., 1997). Furthermore, root removal enhances flowering in the short day plant, *Chenopodium rubrum*. The largest promotion effect is observed when de-rooting coincides with the start of the inductive treatment. However, the effect of de-rooting cannot be attributed solely to cytokinin deprivation (Vondrakova *et al*., 1988).

In cultured tobacco explants, long day induction results in a decrease of the auxin level at the 16th hr in the apical bud. Thus, the auxin and cytokinin ratio is decreased in the apical bud of induced plants. The balance between these two hormones is the control of many physiological processes inducing flower formation (Bernier *et al*., 1993). Cold temperature may interrupt polar IAA transport from shoot tips and subsequent increased cytokinin production in root which may finally elevate the level of the hormone in the apical bud of mango (Naphrom, 2004). Treated longan with potassium chlorate also found lower auxin and higher cytokinin values in buds and that might be prerequisites for flower induction (Hegele *et al*., 2004).

Application of GA can induce flowering in some species. However, applied GA is rarely effective in inducing flowering in short-day plants. Moreover, they generally inhibit flowering of woody plants. Even within long-day plants, the same GA can have a different effect in different species. In mango, increased GA activity was found in apical buds at higher temperatures, increasing their sink strength for flushing. It indicates that a low concentration of GA_3 is required for flower induction (Naphrom, 2004). In longan, low level of GA-like substances were found before flower bud initiation (Boonplod, 1996). GA_3 spraying during flower bud induction significantly inhibited the activities of phenylalanine ammonialyase, polyphenoloxidase, peroxidase and IAA-oxidase, and increased the level of indole-3-acetic acid (IAA). GA also delayed the biosynthesis of lignin in leaves of the current shoots, as well as induced the more vigorous growth and inhibiting flower bud initiation and formation (Li *et al*., 2003).

The ethylene insensitive mutants, *ein* 2 is slightly delayed in flowering, and ABA deficient mutants flowering somewhat early under non inductive photoperiods. Therefore, ethylene and ABA should play some roles in floral promotion and repression respectively (Levy and Dean, 1998). However, ethylene probably dose not contribute to the photoperiod control of flowering in *Chenopodium rubrum*; because the flowering response to darkness duration is rhythmic whereas ethylene production is not (Machackova *et al*., 1997). When longan was treated with ethephon, it was found that cytokinin and ABA concentrations were increased but a GA concentration of shoot was decreased. It was concluded that cytokinin and ABA concentration

promoted flower bud morphogenesis while GA inhibited it (Qiu *et al*., 2001). Under natural condition, a period of cool and dry weather is ideal for flowering of longan. It is believed that inductive conditions trigger changes in endogenous hormones. The low temperature and drought promoted the synthesis of ABA in roots and flower in tropical fruits (Bower *et al*., 1990).

6. Potassium chlorate and flowering of longan

6.1 Potassium chlorate property

Potassium chlorate is a compound containing potassium, chlorine and oxygen, with the chemical formula $KClO_3$. In pure form, it is a white crystalline substance. It is the most common chlorate in industrial use. It is usually present in well-stocked laboratory. It is used as an oxidizing agent, to prepare oxygen, as a disinfectant and in explosive and fireworks. Potassium chlorate reveals the physical properties as molecular weight 122.55 g/mol; density 2.32 g/cm^3; melting point 356 $^{\circ}C$; boiling point 400 $^{\circ}C$ and giving of oxygen; solubility in water 7.3 g in 100 ml of water at 20 $^{\circ}C$ (Wikipedia, 2007). Derivative of potassium chlorate is preferably by interaction of solution of potassium chloride and sodium chlorate or calcium chlorate. Potassium chlorate is moderately toxic, form explosive mixtures with combustible materials such as sulphur, sugar, etc. and strong oxidizing agent (Hawley, 1981).

6.2 Effect of potassium chlorate on plant physiology and biochemistry

The toxic effects of chlorate on higher plants and bacteria have been shown to follow the reduction of the relatively innocuous anion, chlorate, to the poison, chlorite. The reducing system responsible for the reduction of chlorate to chlorite has been shown to be nitrate reductase, on the basis of evidence obtained with young wheat plants. Chlorate and nitrate are both substrates for the enzyme, and each is a competitive inhibitor of the reduction of the other. The toxic effect of chlorate on algal growth should reflect the induction and suppression of the enzyme, nitrate reductase.

Nitrate reductase, the first in a series of enzymes that reduces nitrate to ammonia, is sensitive to a number of environmental factors. Activity varies under the influence of light intensity, CO_2 levels, temperature, water availability, and the nitrate supply. When other environmental factors remain constant, nitrate reductase activity appears to be inducible by nitrate.

Potassium chlorate dissociates into potassium ion (K^+) and chlorate ion (ClO_3^-) when dissolves in water. Chlorate, the chlorine analog of nitrate (NO_3^-) is used extremely as herbicide. Plant exposed to chlorate display various system i.e. root growth is severely inhibited and leaves yellow, whiter, and die (LaBrie *et al*., 1991).

Chlorate is a substrate for the enzyme nitrate reductase (NR). Plants were found to be reduced chlorate *in vivo*, and reduction products chlorite (ClO_2^-) and hypochlorite (ClO^-) were shown to be rapidly acting toxins that happened all cell types treated. At high concentration (0.5 $mol.m^{-3}$) and during prolonged exposures, chlorate has proven to be toxic to most plants (Siddigi *et al*., 1992). The toxicity was enhanced by light, but decreased in higher nitrate concentration (LaBrie *et al*., 1991). The induction of BA- and ABA-modulated NR mRNA in nuclear transcription by light is rapid and observable within 10 min of light treatment. It was suggested that light induced gene expression might involve modification of pre-existing factors previously induced by the hormones (Bradford and Trewavas, 1994). The possible explanation that chlorate treatment stimulates the expression of NR gene in *Arabidopsis* is increasing the level of NR and production of chlorite (LaBrie *et al*., 1991). However, chlorite and chlorate fail to induce NR in barley, (*Hordeum vulgare* L.) cv. Klondiket (Siddigi *et al*., 1992). The low level of NR protein and activity may be due to inactivation of NR by chlorite, leading to rapid degradation of the enzyme (LaBrie *et al*., 1991).

Nitrate was reported to be a competitive inhibitor of chlorate influx, and chlorate found to inhibit nitrate influx: possibly from chlorate toxic (Kosola and Bloom, 1996). Siddigi *et al.* (1992) indicated that chlorate uptake was induced by nitrate, but not by chlorate itself. Chlorate and chlorite failed to induce nitrate transport. However, rate of ClO_3^- uptake is substantially lower than rate of nitrate

uptake. The specificity of NR the Km for chlorate is 50 and 100 times greater than for nitrate ((LaBrie *et al*., 1991).

Sritontip *et al*. (2005) found that the plants that were treated with $KClO_3$ had efficiency of photosystem II (Fv/Fm) at the 1st week before terminal bud break. The net CO_2 assimilate rate at the 1st week before terminal bud break and the net CO_2 assimilate rate during terminal bud break and the transpiration rate were higher than the untreated plant. Potassium chlorate had an effect on total nitrogen. It could increase total nitrogen but total nonstructural carbohydrate tended to decrease before flowering (1st-3rd week) then increased after flowering. The contents of cytokinin-like substances were higher but gibberellin-like substance contents were lower in longan cv. Daw before flowering (Wangsin and Pankasemsuk, 2005). Hegele *et al*., (2006) found that $KClO_3$ affected photosynthesis in leaves and endogenous hormone levels in terminal buds of longan and mango. It reduced photosynthesis and auxin but increased cytokinin contents.

6.3 Effect of potassium chlorate on flowering of longan

In longan, about 20 to 25 days after an application of potassium chlorate, inflorescence emerged in most treated trees. However, some trees showed declining symptom such as yellow leaves and some shoot were sprouted in some trees without flowering (Subhadrabandhu and Yapwattanaphun, 2001). Longan trees with mature leaves, about 45 days old, showed the best response to potassium chlorate. The tree should be dormant and healthy at the time of application. Trees growing on sandy soil respond better than those grown on heavy clay soils. Apply potassium chlorate, as soil drench is the most effective treatment to induce flowering in longan. A significant reduction in photosynthesis was observed for about 10 days after the application (Hegele *et al*., 2004). High concentration of potassium chlorate may cause defoliation, restricted growth, panicle malformation and shoot deformity (Manochai *et al*., 1999b).

Chlorate ion is uptaken by xylem in longan trees. It competes with nitrate ion for nitrate reductase. Therefore, the reduction of nitrate is interrupted. Ammonium ion has diminished and amino acid and protein are decreased too. On the other hand,

chlorate ion is reduced by nitrate reductase and chlorite ion is reduced by nitrite reductase. The product that occurred will cause DNA methylation and gibberellin synthesis is later reduced (LaBrie *et al*., 1991).

The effectiveness of potassium chlorate in flowering induction in longan are based on the stage of leaf development, amount of the chemical, duration of application and soil texture (Tapingkae, 1991). Manochai *et al*. (1999a) found that the optimum stage of leaf development was at 45 days old, approximately. The amount of potassium chlorate used depended on the size and age of longan trees. General recommendation for potassium chlorate application was 5-10 g per square meter of canopy by soil drench or 100 – 3,000 ppm in foliar application (Pankasemsuk, 1999). Trees grow in sandy soil responded better than those grown in heavy clay. Potassium chlorate application, as soil drench was the most effective method to induce flowering in longan. A significant reduction in photosynthesis was observed about 10 days after application (Hegele *et al*., 2006).

Besides, Sinlaphasomboon (2007) reported that by gridling, it revealed that potassium chlorate was transported from the root to the shoot of the treated trees via xylem. Therefore, the leaves located above the girdling line still could show the effect of potassium chlorate which applied by soil drenching. By girdling with defoliation, they revealed that leaves played an important role in flower induction process by potassium chlorate. There should be some substances and/or signals which were synthesized from the leaves and transported to the shoot via phloem which played an important role in flower induction process by potassium chlorate. Moreover, it was found that total protein contents of the flowering tree were higher than non flowering tree. In the flowering tree leaves, there were two new groups of protein, molecular weights of 17.18 and 33.88 kDa, synthesized in leaves during the flower induction period. These groups of proteins were composed of some different types of proteins which had the same molecular weight but different in isoelectric changes. However, potassium chlorate did not affect the isozyme patterns of peroxidase, isomerase, shikimic dehydrogenase, malate dehydrogenase, superoxide dismutase and glucose-6-phosphate dehydrogenase.

6.4 Effect of potassium chlorate on hormonal changes in longan

Thoongkeaw (2001) found that gibberrellin in longan cv. Daw treated with potassium chlorate at concentration of 200, 500 and 800 g/tree was higher than untreated tree. The gibberrellin content was high in first week and it was decreased until 6th week after the application. According to Wangsin (2002), who found that the gibberrellin-like substance content tended to be low before flowering. On other hand, the cytokinin-like substance tended to be continuously increased and it was highest in the last week before flowering. As the study of Boontum (2002), it was found that the gibberrellin content (GA_3) was higher in treated tree than untreated tree. The untreated tree had lowest GA_3 in 2 weeks before flower bud observation. Whereas, zeatin content increased and it was highest in 4th week and then it was stable until 3rd week. After flower bud observation, the gibberrellin content in treated tree was lower than untreated tree. zeatin content in both treated tree and untreated tree were low but the treated tree had the lower content and increased in inflorescence extension. Hegele *et al. (*2004) reported that the longan tree flowered in 17th day after potassium chlorate application. The iAdo/iAde (isopentenyladenosine/ isopentenyladenin) increased at 15th day after potassium chlorate application whereas Z/ZR (zeatin/zeatinriboside) content was high at 4 days after that. IAA content (indoleacetic acid) was lowest at 15-19 days after potassium chlorate application. Besides, Kiatsakun (2004) found that auxin, cytokinin and ethylene in root of treated tree were significantly higher than untreated tree at the second week. Auxin content in root had negative correlation with cytokinin, gibberrellin and ethylene. However in untreated tree the auxin content not had no correlation with cytokinin, gibberrellin but it had medium negative correlation with ethylene. Recently, Srikasetsarakul (2007) found that IAA content in shoot and leaf diffusates increased in the vegetative stage. Whereas, IAA leaf diffusate was lower during flowering. However, ethylene content in shoot of longan cv. Daw tended to decrease at 8th, 6th and 4th before flowering, but increased at 2nd week before flowering. This showed that ethylene might be influenced on longan flowering. (Thonglem and Pankasemsuk, 2001).