Chapter 2

Review of Literature

1. The transition of flowering

The changes in developmental primodia initiated at the shoot apical meristem are controlled by environmental and endogenous signals. From the physiological studies have led to three models for controlling the flowering time. The florigen concept was proposed that florigen, a flower-promoting hormone, was produced in the leaves under favorable photoperiods and temperatures to the shoot apex. However, its chemical nature has remained elusive. The second model, the nutrient diversion hypothesis, proposed that inductive treatment result in an increase in the amount of assimilate moving to the apical meristem, which in turn induces flowering. The last model is the multifunctional control model, which proposed that a number of promoters and inhibitors, including phytohormones and assimilates are involved in controlling the developmental transition. According to this model, flowering can only occur when the limiting factors are present at the apex in appropriate concentrations and at the right times (Levy and Dean, 1998). However, factors affecting flower induction are from both external and internal circumstance.

1.1 External factors of flowering

In some species, the timing of flowering is primarily influenced by environmental factors, which include photoperiod, light quality, light intensity, vernalization, nutrient and water availability. Photoperiodic promotion pathway begin with photoreceptors, which initiate signals that interact with a circadian clock and circadian rhythm. Plants detect light in at least five regions of the visible spectrum by using at least three classes of photoreceptors. Blue light and ultraviolet-A are detected by the cryptochromes, red and far-red light are detected by the phytochromes, and ultraviolet-B is detected by an unidentified photoreceptor (Thomas and Vince-

Prue, 1997). In *Arabidopsis* light quality affects flowering time, with red light inhibiting and far-red light promoting flowering. Blue light alone promotes flowering via *CRYPTOCHROME 2* (*CRY2*) and *CONSTANS* (*CO*). The circadian clock is believed to affect the expression of downstream genes that operate in the photoperiodic promotion pathway, including *CO* (Levy and Dean, 1998).

Vernalization promotion pathway, the site of the perception of vernalization is the shoot apex and actively dividing cells. In Arabidopsis, cold treatment leads to specific changes in gibberellin (GA) metabolism led to propose that vernalization causes a specific reduction in cytosine methylation. This reduction, hypothesized, results in the activation of the gene encoding kaurenoic acid hydrolase, an enzyme that catalyzes an early step in GA biosynthesis. Litchi flowering induced by cold temperature, flowering was very weak when day shoot temperatures and root temperatures exceeded 20 ^{0}C. In litchi cv. Tai So, panicle were visible from 3-7 weeks after transfer to low temperatures with emergence earliest at 20 ^{0}C during the day and 12.5 ^{0}C during the night (Menzel *et al.*, 1989).

Some species are less sensitive to environmental variables and appear to flower in response to internal cues such as plant size or number of vegetative nodes. Flowering can also induced by others such as nutrient deficiency and drought. 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). Longan, a subtropical tree grows well in the tropics but requires a prominent change of seasons for satisfactory flowering. A short (2-3 months) but cool (mean temperature 15-22^{o}C) winter season induces flowering (Wong and Ketsa, 1991).

1.2 Internal factors of flowering

Plant nutrition is proposed to be one of an internal factor, strawberry cv. Toyonaka 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). Nevertheless, 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 activates transcription of genes that are involved in nitrate transport (Coruzzi and Zhou, 2001). Expression of NR (nitrate reductase) and NiR (nitrite reductase) genes is induced by nitrate. Nitrate is known to regulate via the intermediate of the glutamine and glutamate ratio, the phosphorylation and activity of PEPase (phosphoenolpyruvate carboxylase), SPS (sucrose phosphate synthase) and NR enzymes that control the distribution of photosynthetic carbon between the synthesis of sucrose and amino acids (Limani 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 *Sinapsis 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 support 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 ^{0}C and 15/20 ^{0}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 strangulation 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 *pgm* (phosphoglucomutase) 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 show that the late flowering phenotype under short day conditions of the *pgi 1-1* (phosphoglucoisomerase) 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 *S. 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 also 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).

There is an evidence from studies on *S. alba* that long-distance 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 ^{0}C (12.5 ^{0}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 lychee and longan, found generally low in total cytokinins and to increase many fold after flower bud initiation (Chen, 1991 and Huang, 1999). In longan, also 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 rurum*. 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.*, 1998).

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 interrupted 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, 2003). 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.*, 2003).

Applying GAs can induce flowering in some species. However, applied GAs is rarely effective at 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 Arabidopsis, signaling by GAs appears to play an inductive role in flowering. The role of GAs in activation the *LEAFY* (*LFY*) promoter has recently been found. GA_3 alone had no effect, sucrose produced a small increase, and both together had a synergistic effect. The precise role of GAs in transition to flowering is unclear (Blazquez *et al.*, 1998). *LFY* is a potent inducer of flowering in dicotyledons (Weigel and Nilsson, 1995). The expression of the GA responsive floral meristem identity gene *LFY* was very low in the vegetative apex of Arabidopsis but increased dramatically after the second day of exposure to long days. *GAMYB* act as the *LFY* promoter (Gocal *et al.*, 2001). 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, 2003). 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 ammonia lyase, polyphenoloxidase, peroxidase and IAA-oxidase, and increased the level of indole-3-acetic acid (IAA). GA also delaying the biosynthesis of lignin in leaves of the current shoots, as well as

inducing 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 flower somewhat early under non inductive photoperiods. Suggesting that a role for ethylene and ABA in floral promotion and repression respectively (Levy and Dean, 1998). However, ethylene probably not contributes 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). Treated longan with ethephon 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. Suggested that GA inhibits flower initiation but promotes differentiation in longan (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).

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 promotes 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). Cytokinin activates 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 effect on development are rather secondary effect than primary effect. So, 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 increase 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 GAs, many down stream genes involve in flower initiation and development.

1.3 Genetics of flowering

Several genes that are required to mediate the transition to flowering were identified in pea. Some of these genes act in leaves to produce a floral stimulus or to inhibit a promoter of vegetative growth, whereas other genes function at the shoot apical meristem to mediate responsiveness to external signals (Colasanti and Sundaresan, 2000).

Studied in *Arabidopsis* found that flowering was involved the sequential action of two groups of genes: those that switch meristem from vegetative to floral (floral meristem identity genes), and those that direct the formation of the various flower parts (organ identity genes). Therefore, genes that control flowering time can be expected to interact with floral meristem identity genes, which in Arabidopsis include *LEAFY (LFY), APETALA 1 (AP 1), CAULIFLOWER (CAL), AP 2,* and *UNUSUAL FLORAL ORGAN (UFO)* (Levy and Dean, 1998). However, an important regulator of the final stages of flowering in Arabidopsis is *LFY* gene (Colasanti and Sundaresan, 2000). The gene encodes a transcription factor, *LFY*, a target of the flowering time regulators, acts at the shoot apical meristem and surrounding leaf primodia to activate *APETELA 1*. The floral meristem identity genes are capable to influencing flowering time. The response of the flowering time mutants to environmental treatments combined with genetic analyses of epistasis, have established the existence of at least four pathways that control flowering time in Arabidopsis. Floral repression pathway(s) may be a built-in mechanism that prevents flowering until the plant has reached a certain age or size. There are many genes involve this process, but *EMBRYONIC FLOWER* (*EMF*) genes have been considered to play a major role in repression of flowering. Methylation may play an important role in the repression of floral transition. Autonomous promotion pathway is believed to increasing antagonize this repression as the plant develops (Levy and Dean, 1998).

2. Potassium chlorate and flowering of longan

The properties of potassium chlorate are transparent, colorless crystals or white powder, cooling and saline taste. Soluble in boiling water, Sp. gr. 2337; mp 368°C; bp, decompose at 400°C and giving off oxygen (Hawley, 1981). Potassium chlorate 7.3 g dissolves in 100 ml of water at 20°C (Dean, 1985). Derivation 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 sulfer, sugar, etc. and strong oxidizing agent. To use this chemical should be careful (Hawley, 1981). Potassium chlorate ($KClO_3$) 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 a herbicide. Plant exposed to chlorate display various systems: root growth is severely inhibited and leaves yellow,

whither, and die (LaBrie *et al.*, 1991). However, plants have protection system, peroxidases are widely distributed in plants, play major roles in the biosynthesis of cell wall polymers, are implicated in wound healing or defense against pathogen and other external attack (Wititsuwannakul *et al.*, 1997).

Chlorate is a substrate for the enzyme nitrate reductase (NR). Plant 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 plant (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 preexisting 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 failed 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. Nevertheless, rate of $^{36}ClO_3^-$ uptake is substantially lower than rate of nitrate uptake (Touraine and Glass, 1997). The specificity of NR: the K_m for chlorate is 50 and 100 times greater than for nitrate (LaBrie *et al.*, 1991).

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 shoots were sprouted in some trees without flowering (Subhadrabandhu and Yapwattanaphun, 2001). Longan tree with mature leaf, 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.* 2003). High concentration of potassium chlorate may cause defoliation, restricted growth, panicle malformation and shoot deformity (Manochi *et al.*, 1999).

3. The roots

Roots serve a multitude of functions. They are responsible for anchorage, supply the plants with water and nutrients, and exchange various growth substances with the shoots. Plant roots respire nearly half of the carbon imported from photosynthetic tissues to provide ATP for use in energy- consuming process such as ion uptake, maintenance and turnover of existing tissue and biosynthesis new tissue (McDonnell and Farrar, 1993). The high percentages of dry matter partitioning to roots of mango at 15/10 and 20/15 °C were 95 and 76 percent respectively. When vegetative growth was minimal, is due to the greater sink strength of roots at low temperature (Whiley *et al.*, 1989).

Roots are capable of synthesizing the major groups of phytohormones. Cytokinins are strongly indicate as root factors which are transported via the xylem to the shoot, where they exert a major regulatory influence on growth, photosynthesis, and timing of senescence. It is well established that the meristematic cells around the quiescent center at the root tip as well as the root cap are the major sites of cytokinin localization. Root tip or root meristem tissue may be the site of cytokinin synthesis (Bernier *et al.*, 1993). Root appears to contain different forms of cytokinins, which in some cases are not identical with those found in xylem sap. There is a diversity of cytokinins among the different plant organs. Seasonal variation of cytokinin content in xylem sap of apple plants appeared to be dependent on the soil temperature. The response of bean roots to heat treatment indicated a dependence of the shoot on root cytokinin for cell wall synthesis and for photosynthesis. This may be a result of the role played by cytokinins in maintaining the ultra structure of cholroplast grana and stroma thylakoids. The effects of water stress on some processes in the shoot are similar to heat stresses, which may be related to a decreased cytokinin level. In sunflower found a 50 percent decrease of cytokinin in shoot but a two-fold increase in the roots. The transportation may be inhibited due to stress. Abscisic acid (ABA) and its precursors, xanthophylls and phaseic acid probably synthesized in the roots. The

increase in the ABA content of the stressed roots correlated well with the increase in stomatal resistance of the epidermis of the leaves. The leaves showed no change in water potential, which indicated that ABA serves as a signal from the root to the shoot under condition of changing water potential in the root medium. Root tips are capable of synthesizing ABA in response to decreasing water potential. Ethylene is capable to produce in all plant tissue, which cannot be transported from organ to organ in significant quantities for its gaseous nature. Therefore, it is believed that the effects associated with root water logging and the rise of ethylene levels in the shoot are due to a signal from the roots than ethylene. However, 1-aminocyclopropane-1-carboxylic acid, a precursor of ethylene occurs in root exudates and its synthesis is enhanced under anaerobic conditions. Indole acetic acid was synthesized in excised roots. Root cap or the quiescent center is involved in IAA synthesis. The level of IAA in roots changes during development. In *Euphorbia escular*, the level of free IAA in primary roots decreased after flowering to 40 % of the level before flowering (Nissen and Foley, 1987). The root may be a site for interconversion of GAs produced in the shoot rather than a primary source. *Phaseolus coccineus* produces GA_{19} in the shoot, then moves to the roots where it is converted to GA_1. In turn, GA_1 is exported back to the shoot. Adverse conditions in the root environment have been shown to affect the amount of GA exported from the root. Low root temperatures reduce the GA content of maize and tomato root exudate, and at the same time markedly restrict shoot size (Chanan and Birnbaum, 1996).

Interactions between hormones are further illustrated by studies of the changes caused by perturbations of the root environment. Under water stress, root of sunflower plants responded by drastically rose the ABA content up to 32-fold as compared with controls, where as cytokinin content of the leaves dropped by half. Qualitative changes occurred in the cytokinins of the stress roots, with a large increase in zeatin-glucoside. Low root temperatures (8-13° C) were shown to reduce the growth of shoot and to lower the GA and cytokinin content in maize root exudate. At the same time, the ABA content of the exudate had increased. When the plant were grown at higher temperatures (18-33° C) the GA and cytokinin content of the xylem sap higher but that of ABA decreased. The assay studies find change in the levels of free and conjugated ABA and in cytokinin in the xylem sap of drought stressed plants (Bano *et al.*, 1994). The finding confirmed the idea that the levels of both hormones change simultaneously on account of stress but in

opposite directions. From the studies on the interaction among hormones, it may be concluded that the regulation of various developmental processes or of the responses to the environment is a consequence of the activities in concert of all hormones. No single hormone has an overriding rule over that of the others. It follows from this that the responses evoked by hormones are rarely proportional to the concentration of any individual regulator (Chanan and Birnbaum, 1996).

Studied in tobacco found that number of nodes produced by the axillary bud on an isolate stem piece can also be increased when roots are present on the stem piece prior to the time of floral determination of the bud meristem. The results are consistent with the interpretation that the roots produce a substance(s) that maintains vegetative growth. Alternatively, the root may produce an inhibitor that prevents the meristem from responding to floral stimulus. It is also possible, however, that roots inhibit floral initiation by acting as a sink for some substance that is required for floral initiation (McDaniel, 1996).